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TONGUE GYMNASTICS
(SEE PAGE 221)

Red-Eyed Chickens

Semi-Sterile Maize

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CONTENTS

A Multiple Sex-Chromosome Mechanism in a Root Maggot
By J. W. Boyes 195

A Red-Eye Mutation in White Plymouth Rocks
By E. Roberts, W. E. Shaklee and H. F. Falls 201

Genic Control of Semi-Sterility in Maize
By Oliver E. Nelson, Jr., and Gerald B. Clary 205

Variations in Mitosis
By G. B. Wilson, TeMay Tsou and Peter Hyypio 211

Studies on the Mutagenicity of Phenol in Drosophila Melanogaster
By Frank M. Sturtevant, Jr. 217

Tongue Rolling and Tongue Folding and Other Hereditary Movements of the Tongue
By Edward E. Gabres 221

Electron Microscopy of Salivary Gland Chromosomes of Drosophila
By Irwin H. Herskowitz 227

Chromosome Numbers in Coleus
By N. S. Reddy 233

High and Low Thyroidal Response to the Feeding of Thiouracil to New Hampshire Chickens
By William E. Shaklee and C. S. Shaffner 238

C-Mitosis in Commelina Communis L.
By C. A. Berger, A. L. LaFleur and E. R. Witkus 243

The Inheritance of Growth Rate and Efficiency of Gain in Milking Shorthorn Steers
By M. L. Kohli, A. C. Cook and W. M. Dawson 249

Notes and Reviews

Haploid and Triploid Sorghum
By Harold J. Kidd 204

Origin of Life (Rev. of Hyndman: *The Origin of Life and the Evolution of Living Things: An Environmental Theory*)
By Oscar Riddle 215

Heredity in Uterine Cancer (Rev. of Murphy: *Heredity in Uterine Cancer*)
By George W. Woolley 216

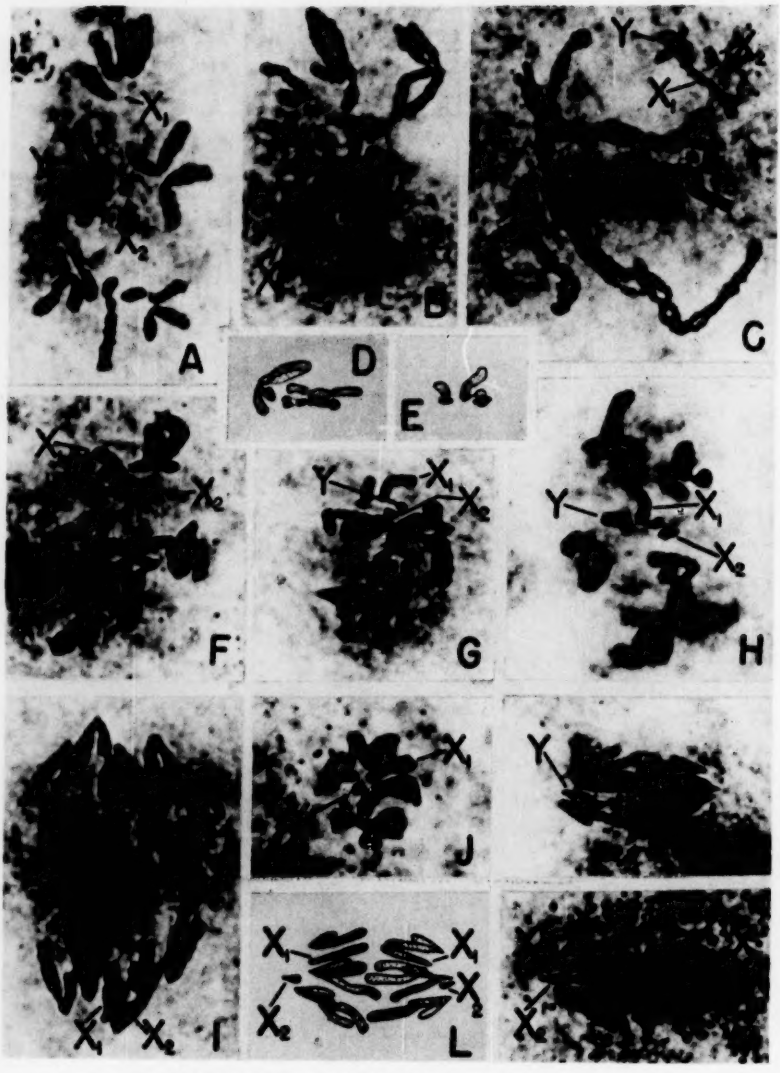
Zebra Finch Mutations
By Matthew F. Bender 220

Correction *in re* the Tomato Genetics Cooperative and the American Tomato Yearbook 232

Disease Resistance from a Wheat × Rye Cross
By N. F. Jensen and G. C. Kent 242

Frontal Hair Direction in American Chinese, Indian and Negro Populations
By Vilhelm Kul 247

The Problem of the Pumpkin-Rind Watermelon
By Jay M. Gordon 252



SEX-CHROMOSOME MECHANISM OF *HYLEMYA FUGAX*

Figure 1

A—Shows the somatic complement in larval ganglion of the male. *B*—The same in larval ganglion of the female. *C-M*—Meiosis in males. *C*—Diplotene. *D*—The X_1 *Y* X_2 trivalent in late metaphase I. *E-I*—Stages in anaphase I disjunction of the sex-chromosome trivalent, (see text for explanation). *J*—Prophase II. *K*—Anaphase II in a cell containing the *Y*. *L* and *M*—Both anaphase II from the same cell containing X_1 and X_2. All photomicrographs and drawings at 1,800×.

A MULTIPLE SEX-CHROMOSOME MECHANISM

In a Root Maggot, *Hylemya fugax**

J. W. BOYES
Department of Genetics, McGill University

MULTIPLE sex-chromosome mechanisms have now been described in a variety of insects.[4, 10, 11, 16, 18, 20, 21, etc.] However, the only case of an $X_1 X_2 : Y$ type previously seen in *Diptera* was reported by Dobzhansky[7] in *Drosophila miranda* Dobzh. The unusual interpretation of disjunction which he advanced was challenged by Darlington[6] on theoretical grounds and following investigations by Dobzhansky[8], Koller[12] and MacKnight[13], a thorough analysis by Cooper[3] finally established the true nature of pairing and disjunction in the $X_1 Y X_2$ trivalent. In the present case, which also involves an $X_1 Y X_2$ trivalent, all three chromosomes are distinct in size and morphology. Accordingly each of the sex chromosomes can be recognized at many stages of mitosis and meiosis and their behavior in disjunction clearly establishes how the mechanism operates.

Materials and Methods

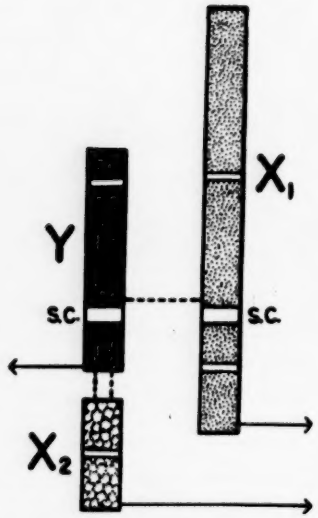
Flies of the species *Hylemya fugax* (Meig.) (*Diptera;* Family Anthomyiidae) are of considerable economic importance in Europe. The specimens studied were obtained as third instar larvae by Mr. George Wishart of the Dominion Parasite Laboratory at Belleville, Ontario, from Wye in England. Somatic chromosome complements were observed in the ganglia of these larvae and to some extent in oogonial divisions in pupae. In late pupal stages the sexes can be distinguished with ease since the males have holoptic eyes and there are distinct sex differences in the genitalia. Aceto-carmine stain was used in all cases in conjunction with Wilson's Venetian Turpentine[22] in a modification of Wilson's procedure, which will be described in detail elsewhere.

Somatic Chromosome Complements

The inspection of the somatic complements found in the ganglia of third instar larvae led to the conclusion that a multiple sex-chromosome mechanism operates in this species. Seven larvae had 13 chromosomes in their somatic complements (Figure 1*A*) each consisting of five pairs of metacentrics, a long acrocentric (X_1), a small dot-shaped acrocentric (X_2), and an intermediate-sized acrocentric chromosome (Y) which is distinctly heterochromatic at metaphase. Fourteen larvae had 14 chromosomes in their somatic complements (Figure 1*B*) each consisting of five pairs of metacentrics, a pair of long acrocentrics (X_1), and a pair of small dot-shaped acrocentrics (X_2). No exceptional complement was found in any cell of any individual. It seemed logical to conclude that males of this species possess five pairs of autosomes $+X_1+X_2+Y$ whereas females possess the five pairs of autosomes $+X_1X_1+X_2X_2$. This was later confirmed by studies of the somatic complements in oogonial divisions (and in meiotic divisions as reported later) in pupae where the sexes are quite distinct morphologically as mentioned above.

Several of the metacentric pairs are distinguishable from other pairs on the basis of location of secondary constrictions (the writer applies the term secondary constriction to achromatic bands extending across the chromosomes, usually at points of decreased diameter) and size. Probably all pairs could be distinguished by careful study. These differences will be discussed in another report, but it is pertinent to mention here that in the smallest metacentric autosome the entire short arm is

*The service rendered by Mr. George Wishart, to whom my sincere thanks are due, is indicative of the fine co-operation and help which the writer has received regularly from the officers of the Dominion Parasite Laboratory, of the Division of Entomology, Science Service, Canada Dept. of Agriculture, in this and other studies. Thanks are also due to my colleague Dr. H. M. Slatis for dissecting the pupae and to the McGill University Research Fund for financial assistance.



SEX-CHROMOSOME TRIVALENT OF *HYLEMYA FUGAX*

Figure 2

Diagram of metaphase I organization of the X_1YX_2 trivalent. The Y chromosome is shown by solid black, X_1 is plain stippling and X_2 circular stippling. The diagram is drawn to scale as measured in early anaphase except for the smaller constriction in the Y which was only seen in prophase. S.C. means prominent secondary constriction; arrows indicate positions of kinetochores and directions of disjunction.

distinctly heterochromatic at metaphase. The long acrocentric chromosome (X_1) has a distinct secondary constriction a short distance from the nearly terminal kinetochore, and in some cases another secondary constriction can be seen in a sub-medial location. The X_2 chromosomes have also a medial secondary constriction discernible in some cases at metaphase. This appearance at somatic metaphase is probably due to the precocious separation of the two chromatids. However, sub-medial constrictions are sometimes observable at anaphase in these chromosomes, and these seem to be true secondary constrictions. Neither the X_1 nor the X_2 is noticeably heterochromatic at metaphase in contrast to the Y chromosome which is regularly heterochromatic at this stage of mitosis. The Y chromosome has a distinct sub-medial secondary constriction. It will be observed from the above and from the examination of Figures 1*A* and *B* that the X_1 and X_2 and Y chromosomes can be identified accurately and with relative ease in this species.

In somatic prophase in males no tendency was found for the X_1, X_2 and Y to be associated. In the females, however, one (or both) of the X_2 chromosomes lies fairly close to the kinetochore end of the X_1 pair in a majority of the cells examined, though in some cases the X_1 and X_2 are widely separated. The same situation was found in certain cells at metaphase (see Figure 1*B*). Since the number of cells in which this point was specifically checked at metaphase was not great (*22*) this tendency may be more apparent than real. Possibly the X_1 and X_2 chromosomes occupy proximal locations in the metabolic nucleus or the small X_2 chromosomes tend to be centrally located on the metaphase plate whereas the long arms of the X_1 chromosomes are displaced toward the periphery of the cell leaving them pointing toward the center. On the whole one is left with the impression that X_1 and X_2 chromosomes tend to be together more frequently than expected on a chance basis.

Meiosis in Spermatogenesis

The sexes were equal in number (12 ♂ :12 ♀) in the pupae used for the study of meiosis. After about two weeks pupation males had large, well developed testes which changed from white to bright orange as meiosis progressed. Abundant meiotic divisions were found in the testes of the 12 male pupae dissected. However only oogonial divisions were found in the ovaries of female pupae dissected at the same time.

Some very early prophases of meiosis were found. Unfortunately, these figures were indistinct and yielded nothing of value in interpreting the pairing tendencies of the sex chromosomes.

The sex chromosomes were first clearly observed in diplotene (Figure 1*C*). Earliest stages show all three together but without consistent orientation in relation to each other. Later the X_1 and Y establish contact at a point near their conspicuous sub-medial secondary constrictions (marked s. c. in Figure 2) but their longer segments extend in various directions from this point (see Figure 1*C*). These contact points do not look like chiasmata. At this time the X_2 lies in the vicinity of the X_1 or of the Y but often more than its own length away. The frequency of the latter situation was relatively high at this stage as seen in Table I.

None of the sex chromosomes are noticeably heterochromatic at this stage, though they are apparently contracting rapidly. A prominent sub-medial secondary constriction (s.c.) separates the two main segments of the X_1, each of which has a less prominent secondary constriction as shown in Figure 2. The X_2 has a distinct, nearly medial, secondary constriction. The Y, in addition to the prominent secondary constriction (s.c. in Figure 2) has a less prominent secondary constriction located sub-terminally. These constrictions are par-

ticularly useful in distinguishing the parts of these chromosomes when they are close together.

Many cells were seen at the diakinesis stage. The observations recorded in Table I show that the X_1 and Y have conjoined in nearly all cases but the X_2 is more than its own length away from the kinetochore region of both the X_1 and Y in over a third of these cases. The X_2 appears to approach the kinetochore region of the short segment of the Y directly and accordingly has no constant position in relation to the proximal segment of the X_1. Thus, when the long distal segments of the X_1 and Y lie in opposite directions, the X_2 lies near the short segment (kinetochore region) of the Y and even when these long segments are more or less parallel the X_2 often does not lie adjacent to the short X_1 segment. This behavior indicates that (during squashing) the Y, with the X_2, may rotate about the point of contact of the Y with the X_1. It also indicates that the X_2 is primarily attracted to the Y rather than the X_1. In general it seems clear from these observations that the sex chromosomes are conjoining to form the trivalent long after conjunction of the autosomes to form bivalents.

The autosomes have appreciably shortened by diakinesis and in some cases repulsion at the kinetochore is apparent. They still appear to be held together at a few points at intervals along their length as at diplotene (see Figure 1*C*) but have lost most or all of their relational coiling. These contact points merely look as if the chromosomes were adhering together; they do not look like chiasmata. Also, the positively heterochromatic arms of the small bivalent continue to repel each other strongly, as first seen in diplotene (Figure 1*C* right center), and continue to do so until they separate at anaphase. It is also noteworthy that, though the distance between kinetochores in these autosomal bivalents seems to increase steadily from diakinesis on, so far no good metaphase plate has been seen.

Figure 1*D* shows the small metacentric pair of chromosomes (which have the heterochromatic short arms) and the X_1 Y X_2 complex in a cell in which the other pairs had just started to separate at the kinetochores. The writer and two of his colleagues independently, saw what they interpreted as two fine strands connecting the X_2 with the proximal short segment of the Y chromosome. The appearance of the interzonal connections between the X_2 and the Y in Figure 1*G* supports this observation. However this point needs further confirmation.

Stages in anaphase I are shown in Figure 1*E* to *I* inclusive. (Figures 1*F*, *G* and *H* might be interpreted as "premetaphase stretch" stages, but the writer prefers to interpret them as true early anaphases.) In Figure 1*F* the X_2 has disjoined from the proximal segment of the Y, whereas the X_1 and Y are held closely together at one point. Figure 1*G* shows the manner of disjunction particularly clearly. The interzonal connections between the proximal portion of the Y and the X_2 are still clear; the connection between the distal segment of the Y and the X_1 is also quite apparent. In Figure 1*H* the latter connection has broken but remnants of the connections between the X_2 and Y can still be detected. No sign of interzonal connections could be seen in the complex drawn as Figure 1*E*. In many cells studied at this stage the X_2 regularly moved to the same pole as the X_1 in disjoining from the Y.

This method of disjunction is clearly confirmed in such late anaphase I stages as that shown in Figure 1*I*. At one pole six chromosomes, including the Y, can be counted whereas seven chromosomes, including the X_1 and the X_2 are present at the other pole. The heterochromatic nature of the Y is quite apparent in this figure.

Cytokinesis follows soon after anaphase I and apparently the chromosomes pass directly into division II. Prophase II is shown, in a cell containing the X_1 and X_2, as Figure 1*J*.

Cells containing the Y chromosome (Figure 1*K*) dividing equationally in anaphase II are not difficult to distinguish from those containing the X_1 and X_2 (Figures 1*L* and *M*). Six chromosomes were counted going toward each pole in many cells containing the Y. Similarly seven chromosomes were regularly counted going to each pole in many cells containing the X_1 and X_2. Many cells in telophase II were found but the small chromosomes were tightly clumped and could not be counted.

In the writer's opinion these observations establish with resonable certainty that a multiple sex-chromosome mechanism of the X_1 X_2:Y type functions in *Hylemya fugax*.

Discussion

The origin of this sex-chromosome mechanism is obscure. Very little cytological work has been done on the Anthomyiidae. Stevens[19] showed a comple-

TABLE I. Conjunction of X_1, Y and X_2 in Prophase.

	Diplotene	Diakinesis-Metaphase
X_1, Y and X_2 in contact or nearly so.	10	29
X_1Y paired, X_2 more than its own length away from proximal segments X_1 and Y.	20	16(+3 in disjunction)
Not certainly analysable.	2	7

ment of six pairs of chromosomes, including one heteromorphic pair from spermatocytes of *Phorbia brassica*. Metz[15] figured somatic (12) and meiotic (6 pairs) chromosomes of *Homalomya* sp., *Fucellia marina* Macq. and *Ophyra leucostoma* Wied. A small heteromorphic pair can be clearly identified in the drawings of chromosomes from males of *Fucellia marina*, but no heteromorphic pairs were shown in males of the other two species. Frolowa[9] showed a small pair of chromosomes, which she considered to be the sex chromosomes of the female in somatic complements of *Pegomyia geniculata* Bouche. Apparently these species all have 12-chromosome complements, including, in two species, a heteromorphic sex pair, whereas two other species have the *X* and *Y* similar or nearly equal in size. The writer (unpublished) has seen the somatic complements of eight species of *Hylemya* (not including *H. fugax*) and meiosis in one of them. Each of the eight has 12 somatic chromosomes including a small acrocentric pair (presumably sex chromosomes) with members of nearly equal, or equal size in both males and females. One may postulate that the multiple sex-chromosome mechanism of *H. fugax* has been derived from an *XY* system by translocations between the *Y* and an autosomal pair plus the loss of one chromosome. This may be so, but the result should be a type with 11 chromosomes in the male and 12 in the female instead of 13 in the male and 14 in the female as found in *H. fugax*. Perhaps the increase in number arose through nondisjunction involving an autosomal pair of chromosomes (two autosomal pairs of *H. fugax* do look similar in size and morphology) but such ideas are pure conjecture. It seems more likely that a related species will eventually be found with a 14-chromosome complement to which the *fugax* complement is related and from which it might be derived.

Cooper[1,2,3] has not accepted Darlington's[5] conclusion from studies on *Drosophila pseudoobscura* that "reciprocal chiasmata are formed between *X* and *Y* following normal pachytene pairing of the homologous segments and that these chiasmata are either in the short arm or in the long arm of the *Y* or—where metaphase pairing fails—in neither." The demonstration of genetic crossing over between *X* and *Y* chromosomes in *Drosophila melanogaster* by Philip[17] and further genetic evidence from Mather[14] have supported Darlington. However, Cooper[2] has observed in *Olfersia* that "The sex chromosomes, which may be a nuclear diameter apart at diplotene, come together and ankylose in a short interstitial region before the close of diakinesis. Although the resulting bivalent behaves and appears thereafter identical in structure with the sex chromosome bivalent of the male *Drosophila pseudoobscura*, clearly it cannot be provided with chiasmata unless, as is very improbable, condensed chromosomes can undergo crossing over." In the same report he also presented a comprehensive criticism of Darlington's viewpoint including the supporting evidence mentioned above. Further, from an intensive analysis of the mechanism of nonrandom segregation in the X_1 X_2 sex trivalent of *Drosophila miranda*[3] he concluded that "X_1 and X_2 conjoin with *Y* by similar mechanisms not involving crossing over, hence independent of chiasmata." It is not desirable to consider the details of this major difference of interpretation here. However, the following pertinent observations on the sex trivalent in *Hylemya fugax* merit mention in this connection: (1) the time of conjunction of the X_1, *Y* and particularly the X_2 is considerably later than for autosomal pairs, (2) in many cases the sex chromosomes have condensed considerably before conjunction and (3) the manner of conjunction of X_1 with the *Y* appears to be the same as that for the X_2 and the *Y*. In the writer's opinion these observations support the conclusions reached by Cooper as quoted above.

MacKnight[13] has suggested in relation to meiotic pairing of the X_2 and *Y* in *Drosophila miranda* that a heterochromatic region may be involved. In *Hylemya fugax* the heterochro-

matic arms of an autosomal bivalent repel each other at the same time that conjunction takes place in the X_1 Y X_2 trivalent. Of course, the heterochromatic arms may contain a different kind of heterochromatin or have attained a different stage of condensation and accordingly considerations of MacKnight's suggestion are still inconclusive.

The configurations seen to date suggest certain conclusions in relation to the partial homology of parts of the X_1, X_2 and Y. It appears that at least a part of the longer segment of the Y has some attraction for a part of the longer section of the X_1 (see Figure 2). Also, the short segment of the Y apparently has some attraction for the X_2 since it is associated with it in prophase and disjoins from it in anaphase. Though the proximal part of X_1 is often seen near the X_2, this probably results from the attraction of the X_2 for the proximal portion of the Y which lies near the proximal part of X_1 after conjunction, and from its position during disjunction from the Y at the same time that the X_1 disjoins from the Y. However, it is possible that there exists some attraction between X_2 and the proximal portion of the X_1 as suggested above in observations on prophase of somatic mitosis.

Little can be said regarding the location of sex-determining factors of *H. fugax*. It is possible that the heterochromatic Y chromosome carries no factors for sex determination and functions only as a common base for directed disjunction of the X_1 and X_2. Other explanations are equally plausible. However, one can say that present observations indicate that the disjunction system operates efficiently in this species.

The finding of this second case of an X_1 X_2: Y sex-chromosome mechanism in the *Diptera* raises the question of their time of origin in evolutionary sequence. These two cases, one in Drosophilidae of the Acalyptratae, and the other in the Anthomyiidae (Muscidae of Curran) of the Calyptratae, may have a common origin since these two series, or sections, of the *Diptera* are fairly close taxonomically. However, the case of *Drosophila miranda* is unique in the widely studied Drosophilidae and in a total of ten species in the Anthomyiidae which the writer (unpublished) has seen to date, plus five reported by other workers, no other case of this type of multiple sex-chromosome complex was found. Most of these species were in the genus *Hylemya* and though European taxonomists place *fugax* in the genus *Pegohylemya*, these species are apparently fairly closely related to *fugax*. Accordingly on the basis of presently available information, the writer is of the opinion that these two X_1 X_2:Y sex-chromosome mechanisms have arisen independently and recently in evolutionary sequence.

Summary

1. Somatic cells of *Hylemya fugax* males possess 13 chromosomes including an X_1, a Y and an X_2, all three of which are distinct in size and morphology, whereas females have 14 chromosomes including two X_1 and two X_2.
2. During meiosis I prophase stages in male pupae the conjunction of X_1 and Y, followed by X_2 and Y takes place producing a trivalent X_1 Y X_2 sex-chromosome complex.
3. The Y disjoins from both X_1 and X_2 in anaphase of meiosis I so that cells in meiosis II regularly contain either the Y, or the X_1 and X_2.
4. Accordingly male determining sperms contain a Y chromosome and five autosomes whereas female determining sperms contain X_1, X_2, and five autosomes.

Literature Cited

1. Cooper, K. W. *Genetics* 29:537-568. 1944.
2. ———. *Proc. Nat. Acad. Sci.* 30: 50-54. 1944.
3. ———. *Genetics* 31:181-194. 1946.
4. ———. *Jour. Morph.* 89:37-58. 1951.
5. Darlington, C. D. *Genetics* 19:95-118. 1934.
6. ———. *Amer. Nat.* 70:74-75. 1936.
7. Dobzhansky, Th. *Genetics* 20:377-391. 1935.
8. ———. *Jour. Genet.* 34:135-151. 1937.
9. Frolowa, S. L. *Z. Zelf. mik. An.* 8:542-565. 1929.
10. Hughes-Schrader, S. *Biol. Bull.* 85: 265-300. 1943.
11. King, R. L. and H. W. Beams. *Jour. Morph.* 63:289-299. 1938.
12. Koller, P. C. *Jour. Genet.* 38:477-492. 1939.
13. MacKnight, R. H. *Genetics* 24:180-201. 1939.
14. Mather, K. *Proc. Roy. Soc. London, B* 132:308-332. 1944.
15. Metz, C. W. *Jour. Exp. Zool.* 21:213-279. 1916.
16. Payne, F. *Jour. Morph.* 23:331-347. 1912.
17. Philip, U. *Jour. Genet.* 31:341-352. 1935.
18. Raychaudhuri, S. P. and G. K. Manna. *Jour. Hered.* 41:277-280. 1950.
19. Stevens, N. M. *Jour. Exp. Zool.* 5:359-374. 1908.
20. White, M. J. D. *Jour. Genet.* 40:303-336. 1940.
21. ———. *Jour. Genet.* 42:143-172. 1941.
22. Wilson, G. B. *Stain Tech.* 20:133-135. 1945.

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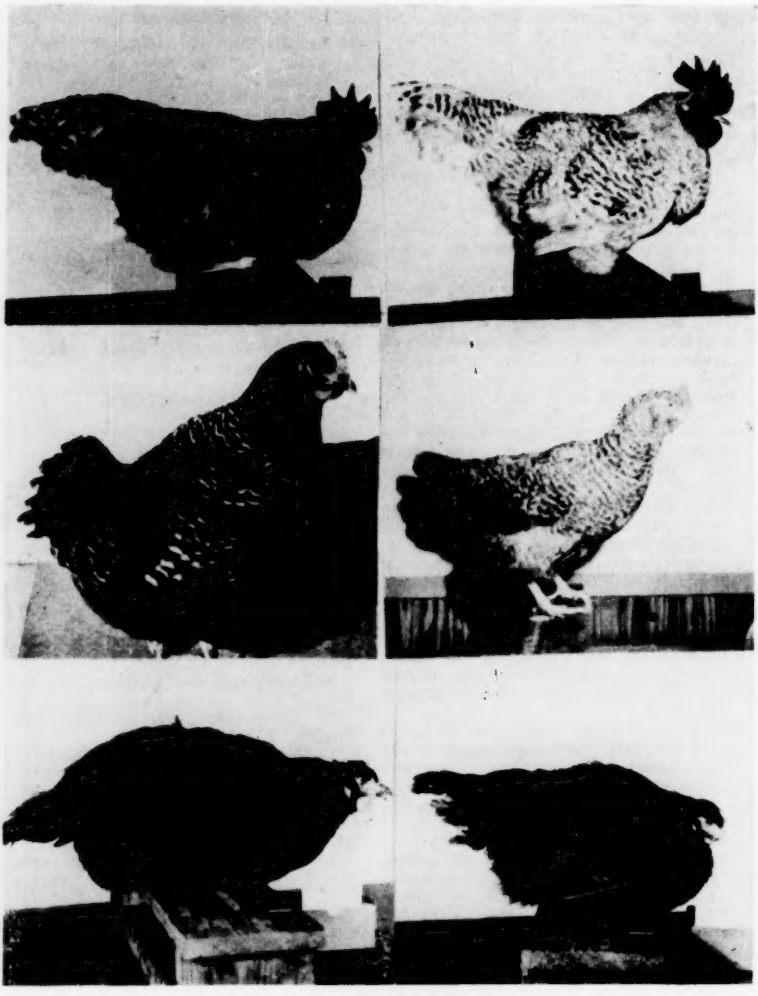


RED-EYE AND NORMAL BIRDS

Figure 3

Normal dark-eyed birds are shown at the left and those with the red-eye condition are shown at the right. Red-eye is recessive to dark-eye. Feather pigmentation is reduced and vision is defective in red-eyed individuals.

A RED-EYE MUTATION

In White Plymouth Rocks

E. ROBERTS, W. E. SHAKLEE AND H. F. FALLS*

THE original red-eyed birds used in this study were obtained from Mr. E. L. Johnson of the Double-R Hatcheries, Olney, Illinois. They came from a closed flock of White Plymouth Rocks of about 150 females and 12 males. The red-eyed chicks were found during two successive years, two or three in each week's hatch. The eyes were darker red than would be expected if an albino mutation were involved.

A red-eyed White Plymouth Rock male was mated by artificial insemination with black Australorp females. The F_1 generation of 73 birds was barred, with the exception of four whites, with a few black or partially black feathers. Two of these splashed white F_1 females mated with an F_1 male produced 70 progeny all of which were dark-eyed. Mated to a red-eyed male, 43 progeny were produced all of which were dark-eyed. We are unable to give a plausible explanation of these results, since all other F_1 females produced both dark and red-eyed individuals.

In Table I the results of matings, excluding the two white F_1 females, are given. Symbols used are *R* for dark-eye and *r* for red-eye. The results indicate that the difference between dark and red-eye is due to a single autosomal gene, red-eye being recessive to dark-eye.

In the heterozygous condition (*Rr*) there is no visible effect on intensity of pigmentation. In the homozygous state (*rr*) the black is reduced in intensity as shown in Figure 3. Red-eye in the rat,[1] and red-eye in Hampshire swine,[4] are also accompanied by reduction in intensity of hair pigmentation.

From observation, vision of the red-eyed birds appeared to be subnormal. They seemed to have difficulty in finding feed and water containers and stayed in the house much more than did dark-eyed birds. Microsections (Figures 4 and 5) of the mutant eye, when compared to the normally pigmented eye, reveal the former to possess melanin in the usual distribution involving both the region of the ciliary body and choroidal coat. A marked reduction in the quantity of melanin pigmentation, however, is observed in the mutant eye. Pigmentation is reduced or nearly absent in the choroid and posterior three-fourths of the globe. It thus appears that the mutant eye is capable of producing melanin pigment, but only in a markedly reduced amount—a dilution factor. It is this lessened quantity of pigmentation which gives the mutant eye the red appearance. There is no evidence of altered anatomy, with the exception of diminution of pigment, which would account for impaired visual acuity. However, albinism in man results in decreased visual acuity, a dislike of light, tearing of the eyes, and a distinct photophobia.

In general, viability of red-eyed mutants is less than that of normal birds. In Table II are given the percentages of mortality through four months of age. Both dark and red-eyed chicks from a given cross were distributed at random in brooders and pens and therefore subjected, in general, to similar environmental conditions. Also, the two matings, *Rr* × *rr* and *rr* × *rr* were hatched at the same time and reared under similar conditions. The apparent defective vision may have had an influence on viability.

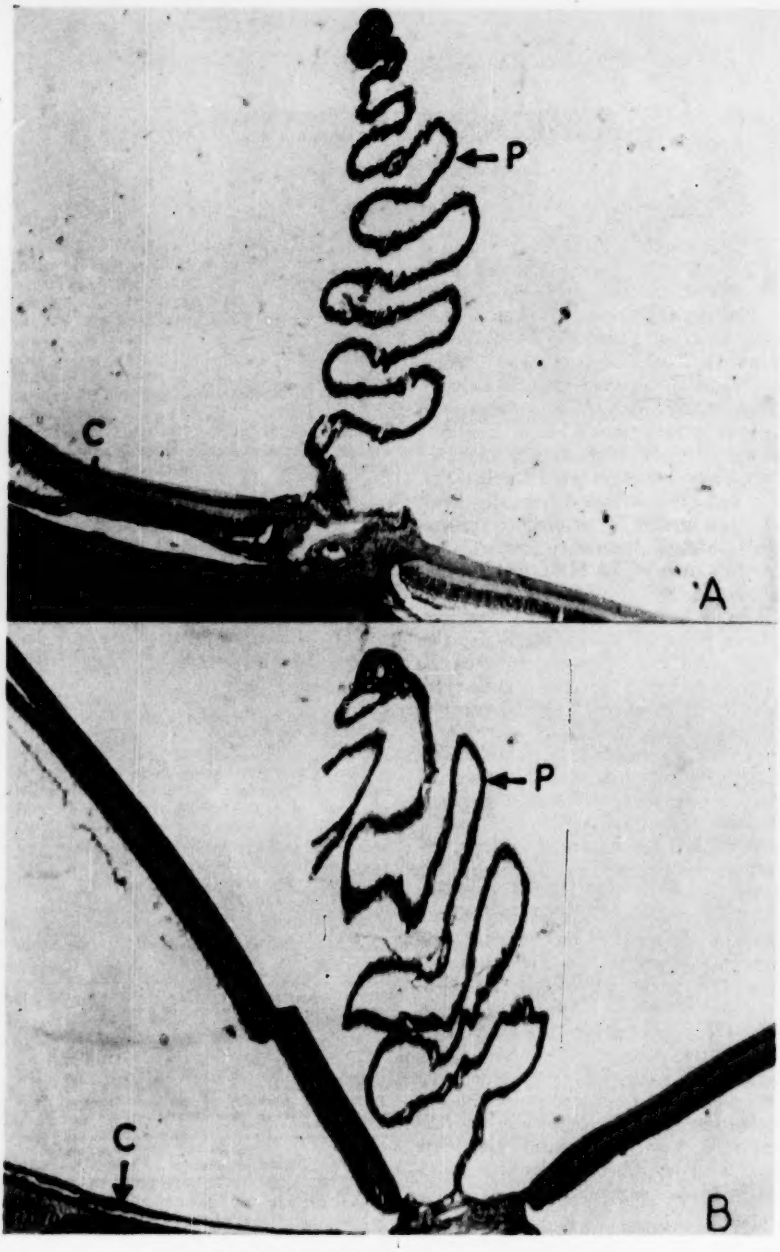
TABLE I. Various matings involving the red-eye gene.

Mating	Number	Dark eye	Red eye	D	D/S.E.
Rr × *Rr*	337	245	92	7.75	.97
Rr × *rr*	135	76	59	8.5	1.46
rr × *rr*	147	—	147	—	—

TABLE II. Mortality of red and dark-eyed birds through four months of age.

Mating	Dark eye		Red eye	
	Number	% mortality	Number	% mortality
Rr × *Rr*	245	11.8	92	20.7
Rr × *rr*	76	13.2	59	37.3
rr × *rr*	—	—	147	58.5
Totals	321	12.2	298	42.6

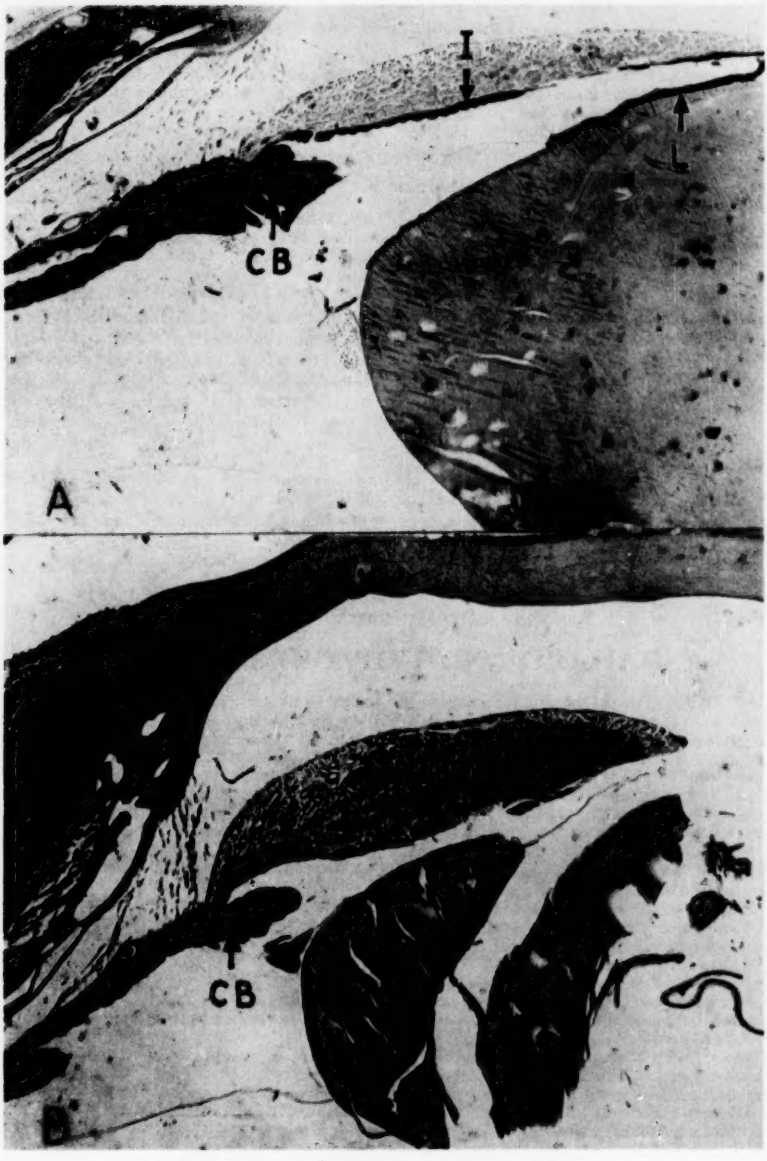
*Department of Animal Science, University of Illinois, Urbana; Bureau of Animal Industry, U. S. Department of Agriculture; and Department of Ophthalmology, University of Michigan, Ann Arbor, respectively.



MICROSECTION OF NORMAL AND RED-EYES

Figure 4

A—Microsection of a normal fowl eye. The pecten (P) and choroid (C) exhibit normal heavy melanin deposition (P = pecten and C = choroid). *B*—Microsection of mutant red-eye. The pecten and neighboring choroid are almost entirely lacking in melanin pigment.



NORMAL AND REDUCED PIGMENTATION

Figure 5

A—Microsection of a normal fowl eye through ciliary body (CB), iris (I) and lens (L). The heavy pigmentation of the ciliary body and posterior iris surface is evident. *B*—Microsection of mutant red-eye through anterior ocular segment. Melanin pigment is normally distributed in the ciliary body and posterior iris surface, but in reduced quantity.

Warren[5] reported a case of albinism in White Wyandottes. In a cross with colored birds, the F_1 generation had normal dark eyes. In a backcross with pink-eye, all pink-eyed birds had white plumage. This apparently is a case of complete albinism which is recessive. Warren[6] reported a pink-eye condition in White Plymouth Rocks due to a recessive gene. In crosses with colored birds the recombination of the pink-eye with color resulted in reduced pigmentation of the plumage. This "pink-eye" is probably analogous to the "red-eye" which we have studied. The term "red-eye" has been used because the eyes are not as pink as seen in albino forms in other animals. In rats the red-eye mutant is distinguishable from the pink-eye mutant, the former being darker red in color.

Mueller and Hutt[3] reported a sex-linked imperfect albinism in Barred Plymouth Rocks, resulting in greatly reduced pigmentation and has been described as "ghost-barred." Two other similar mutations were reported by Hutt and Mueller.[2] One occurred in Barred Plymouth Rocks in Massachusetts and the other in S. C. White Leghorns in Indiana.

The red-eye mutation which we have studied is autosomal and recombines at random with the genes for barring, non-barring and white which were present in the cross.

Summary

The red-eye mutation is recessive to dark-eye. The red-eyed birds are less viable than normal birds. They also seemed to have defective vision.

Literature Cited

1. Castle, W. E. and Sewall, Wright. *Carnegie Inst. Wash. Pub.* No. 241. 1916.

2. Hutt, F. B. and C. D. Mueller. *Amer. Nat.* 77:181-184. 1943.

3. Mueller, C. D. and F. B. Hutt. *Jour. Hered.* 32:71-80. 1941.

4. Roberts, E. and J. L. Krider. *Jour. Hered.* 40:306. 1949.

5. Warren, D. C. *Jour. Hered.* 24:379-383. 1933.

6. Warren, D. C. *Jour Hered.* 31:291-292. 1940.

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HAPLOID AND TRIPLOID SORGHUM

Brown[1] reported haploid S. *vulgare* in 1943. The metaphase plate was usually recognizable and few haploid cells contained bivalents. Diploid cells were observed occasionally in the pollen mother-cells. Gaines and Aase[3] and Levan[4] observed di- and poly-ploid cells in haploid plants, although all chromosomes behaved as though they were in haploid condition, univalents remaining the rule.

Chin[2] observed tetraploid and octoploid *S. vulgare* and reported chiasmata similar in percentage in polyploids to the diploid.

The haploid plant of the present report was one member of a twin seedling of *S. vulgare* var. Resistant Wheatland (G.C. 38288). The second member of the twin was weak and diseased, never reaching maturity or flowering. The triploid plant came from an F_3 population (*ms #2* Dwarf Tan Kafir × Redlan F_3), being observed to be sterile. Since the male sterile (*ms*$_2$) character is inherited as a recessive, the condition was investigated.

All preparations were stained with aceto-carmine in temporary smears of pollen mother-cells.

Results and Discussion

In the haploid plant, from a total of 77 cells observed, 55 were haploid and 22 were diploid. Of the haploid cells, 36 had ten univalents, eight had eight univalents and one bivalent, four six univalents and two univalents, and one presented seven univalents and one trivalent. All were observed at metaphase I or diakinesis. The diploid cells appeared to be abnormal in that individual chromatids were more noticeable than normal and a "stickiness" was observable at anaphase I. These diploid cells differed from the "syncytes" of Levan[4] and Gaines and Aase[3] in that the chromosome behavior resembled diploid, rather than haploid conditions. Fusion of the nuclei must have occurred before chromosome pairing was complete, as pairing appeared to be the rule rather than the exception. The stain of the diploid cells was considerably poorer than the haploids of all stages.

The triploid plant behaved quite similarly to the triploids previously reported. From a total of 24 cells, eight cells had ten trivalents; five had nine trivalents, one bivalent, and one univalent; six had eight trivalents, two bivalents, and two univalents; three had seven trivalents, three bivalents, and three univalents; and one cell demonstrated each of the configurations six trivalents, four bivalents, and four univalents; and four trivalents, five bivalents, and eight univalents.

The haploid and triploid cells were essen-

(*Continued on page 225*)

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GENIC CONTROL OF SEMI-STERILITY IN MAIZE*

An Inbred with Pollen Semi-Sterility and Ovule Semi-Sterility caused by Different Genes

OLIVER E. NELSON, JR. AND GERALD B. CLARY

DECREASES in male and female fertility caused by the abortion or non-production of varying percentages of the potential gametes have received considerable attention from geneticists and cytologists. The role of structural heterozygosity in inducing partial sterility has been elucidated by a number of workers. Starting with the report by Beadle and McClintock[3] that a case of nearly complete asynapsis in maize was controlled by a single recessive gene, the roles of many other genes in mediating meiotic processes have been shown for a number of plant materials. Chittenden and Pellew[5] first demonstrated an extra-chromosomal influence on fertility by showing that in flax a gene for male sterility expressed itself only in a particular cytoplasm. Rhoades[11] later found in maize that a type of pollen sterility was cytoplasmically inherited with nuclear constitution having no effect. Lastly, environmental influences such as high temperatures (Pao and Li[10]) may be decisive in altering the meiotic process and thus causing some degree of sterility in plants which are otherwise normal. Clark[6] in an investigation of ear semi-sterility in hybrids and pollen semi-sterility in inbreds found that chromosomal aberrations, gene-controlled meiotic abnormalities, and environmental influences were all predisposing factors in different cases.

It should be noted that not all gene-controlled forms of sterility are associated with meiotic abnormalities. Beadle[1] in a study of a number of male-sterile genes in maize found that in one line degeneration was initiated as early as the synizetic stage of meiosis while in another line after a normal meiosis the pollen grains were almost mature before degeneration. The times of degeneration of the pollen grains or pollen mother cells in other *male sterile* lines were between these extremes.

In contrast to such genes as *asynaptic* which affect the development of both megaspores and microspores many of the genes responsible for degeneration or meiotic abnormalities affect only one type of gametes. Examples of this unilateral gametic development are the *male sterile* genes mentioned above where the ears develop normally. Other examples of this are the *variable sterile* genes of Beadle[1] and the *pollen abortion* gene of Burnham[4] where the developing microspores alone are affected. Such genes as the *lethal ovule* factors reported by Singleton and Mangelsdorf[13] and Clark[6] cause the abortion of any megaspores in which they are present but are transmitted through the microspore. The present discussion concerns a line of maize displaying both pollen and ear semi-sterility with each type of sterility being due to a single factor which has no influence on the development of the other type of gamete.

Genetic Data

In 1945 it was noticed that in several inbred lines homozygous for *miniature seed* (Lowe and Nelson[8]) approximately one-half of the plants had semi-sterile ears. Subsequent observation of the progeny of one of these selfed semi-sterile plants showed again that one-half of the plants had semi-sterile ears but that all of the plants had about 50 percent defective pollen. This second observation made it difficult to interpret the results on the basis of

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possible structural heterozygosity as was first done.

In 1947 a number of plants of two lines of *mn/mn* in which pollen sterility had been observed were pollinated in the following manner: the silks of each plant were divided in half and one-half pollinated by its own pollen; the other half were pollinated by pollen from a tester stock which was homozygous for the genes, *lm lr f*. At the same time pollen from the *mn/mn* parent was used to fertilize a plant of the *lm lr f/lm lr f* tester. Ready separation of the selfed and crossed kernels in the ears from the *mn/mn* parents was possible because of the defective endosperm type of the *mn/mn/mn* kernels. In 1948 the three progenies for each parent—selfed, *✓ lm lr f* and *✓ mn lr f*—were planted in adjacent rows and scored for pollen sterility and ear semi-sterility. The results are summarized in Table I. Only the *mn/mn* plants on which complete parent and progeny data were available are included. From these data the following conclusions have been reached: the mean of the percent empty pollen for the selfed progeny of each parent plant approximates the percent of empty pollen of the parent; none of the crossed progeny for any plant shows a significant number of empty pollen grains; when the parent plant had a semi-sterile ear, about one-half of the selfed progeny had semi-sterile ears as did also the progeny of the cross in which the *mn/mn* plant was used as the male parent while the reciprocal cross did not show any ear semi-sterility.

These data gave the first clear-cut indication that pollen and ear semi-sterility might be attributable to different causes. They are best explained on the basis that ear semi-sterility is due to the action of a factor which leads to the non-functioning of any ovule which carries it but which has no effect on the pollen and, further, that partial pollen sterility is due to the action of a recessive gene or genes which cause the abortion of a certain percentage of pollen but have no effect on the ovule. Such an ovule factor, *lethal ovule (lo)*, has been reported by Singleton and Mangelsdorf[13] in maize. Plants heterozygous *+/lo* have semi-sterile ears and transmit *lo* only through the pollen since ovules carrying *lo* are abortive. The selfed and reciprocally-crossed progeny of a heterozygous plant would act as did the progeny of the semi-sterile ear parents in Table I, (i.e. the selfed and the crossed progeny where the plant carrying *lo* was used as a male would show approximately one-half the plants with semi-sterile ears while the crossed progeny where the *lo/+* plant was used as the female parent would have no plants with semi-sterile ears). Further, no plants with normal ears would be capable of transmitting the lethal ovule factor. This expectation was realized in both F_2 and backcross progenies of crosses of *mn/mn ✓ lm lr f* as shown in Table II. Backcross progenies show normal ears when the male parent is normal, and some semi-sterile ears when the male parent is semi-sterile in ear type. Selfed progenies have some plants with semi-sterile ears only if the parent had a semi-sterile ear. Both backcross and selfed progenies have approximately the same percentage of plants with semi-sterile ears as would be expected where ear semi-sterility is due to such a lethal ovule factor. It should be noted that the semi-sterile classes generally fall short of the 50 percent expected which may indicate that male gametes carrying this ovule factor may not be effective-

TABLE II. Backcross and F_2 progenies of crosses of *mn mn ✓ lm lr f* showing the segregation of plants with semi-sterile ears as dependent upon the ear type of the male parent.

Pedigree	Ear Type of Male Parent	Plants with Normal Ears	Plants with 50 Ears	% Plants with 50 Ears
(*lm lr ✓ mn* L-7-3 *✓ mn* L-7-3	SS	141	86	37.9
Bulked Backcrosses	Normal	196	0	0
(*lm lr ✓ mn* L-7) F_2 2	SS	78	72	48
(*lm lr ✓ mn* L-7) F_2 11	SS	92	57	38.1
Bulked Selves	Normal	1139	0	0

TABLE I. Phenotypes of selfed and crossed progenies of partially pollen-sterile *mn mn* plants from lines showing ear semi-sterility, 1948.

Plant	Parent % Empty Pollen	Ear*	Range†	Selfed ✓ % Empty Pollen	Ears*	Progenies *mn ✓ lm lr f* ✓ % Empty Pollen	Ears*	*lm lr f ✓ mn* ✓ % Empty Pollen	Ears*
mn L-1	70	SS	17-60	60	14+ 288	0	24+	0	34+
mn L-2	0	NN	0-20	2	24+ 288	0	16+	0	7+ 456
mn L-4	10	+	0-55	20	6+	0	14+	4	13+
mn L-5	10	+	0-60	27	14+	0	9+	0	15+
mn L-7	65	SS	17-70	60	14+ 188	0	15+	0	10+ 1188
mn L-9	10	+	0-40	17	13+	0	11+	0	24+
mn L-10	10	+	10-75	47	2+ 188	0	11+	0	9+ 555
mn L-11	10	+	17-45	25	4+ 756	0	9+	0	7+ 765
mn L-13	10	SS	0-60	22	6+ 569	0	6+	5	5+ 288
mn L-14	5-10	+	10-35	29	15+	0	14+	0	8+

* A "+" indicates a normal ear and "SS" a semi-sterile ear.
† Range of percentage of empty pollen.

ly competitive with those carrying the normal allele. It is unlikely that this is the same lethal ovule factor reported by Singleton and Mangelsdorf[13] since in appropriate backcrosses, *su* Lo_2/Su $lo_2 \times$ *su* Lo_2, it gives no evidence of being linked with *sugary*. They had found that the *su* and *lo* loci were closely linked with two percent recombination. This lethal ovule factor is not linked to *br* on chromosome 1 as shown by F_2 data. This factor will be designated as *lethal ovule*$_2$ (lo_2).

In contrast to the ear semi-sterility, the partial pollen sterility of the *mn/mn* parents was not expressed in the F_1 progenies of crosses with *br/br* lines as shown in Table I. The percentages of pollen semi-sterile plants in backcross and F_2 progenies (Table III) suggest that the semi-sterility is conditioned by a single recessive gene although the numbers of partially male-sterile plants in both F_2 and backcross progenies are significantly below the expected numbers in every case. Further, within either the F_2 or the backcross progenies, the deviations from expectations are rather constant. It may be an indication either of differential fertilization or of incomplete penetrance of the gene in question. Differential fertilization seems unlikely since the first line of Table III shows the segregation for a backcross progeny made with the partly pollen-sterile *mn/mn* parent as the male parent. Here there should be no possibility of differential fertilization, and the percentage of plants with more than 10 percent empty pollen is similar to that in backcrosses where the F_1 plant is used as the male parent. The possibility that there is a cytoplasmic component of this type of partial pollen sterility would seen to be ruled out by the data in Table III, since there is no difference between reciprocal crosses.

The percentage of empty pollen grains in the *mn/mn* lines is subject to considerable genetic modification, as would be expected if due to a factor with incomplete penetrance. This can be inferred from Table I where it may be seen that the mean percentage of empty pollen in the progeny of any selfed plant is well correlated with the percentage of empty pollen of that plant. This is amplified by the data in Table IV showing that lines can be effectively selected for high and low percentages of empty pollen. Further, where the F_2 progeny of a high pollen-sterile plant (*mn* 1-7 with 40 percent empty pollen) are compared with the F_2 progeny of a low pollen-sterile plant (*mn* 1-5 with 10 percent empty pollen) in Table III, it can be seen that the percentages of F_2 plants with some pollen abortion are similar.

It has been noted in referring to Table I that ear semi-sterility appears in certain F_1 progenies unaccompanied by pollen sterility. In backcross and F_2 progenies all four possible types of plants are recovered. For example, in the 1950 backcross progeny data, there were 91 pollen and ear normal plants, 40 pollen semi-sterile and ear normal plants, 58 pollen normal and ear semi-sterile plants, and 28 pollen and ear semi-sterile plants. These data show not only that the factors for each type of semi-sterility are not dependent upon each other for expression but that there is no linkage between the factors.

Cytological Data

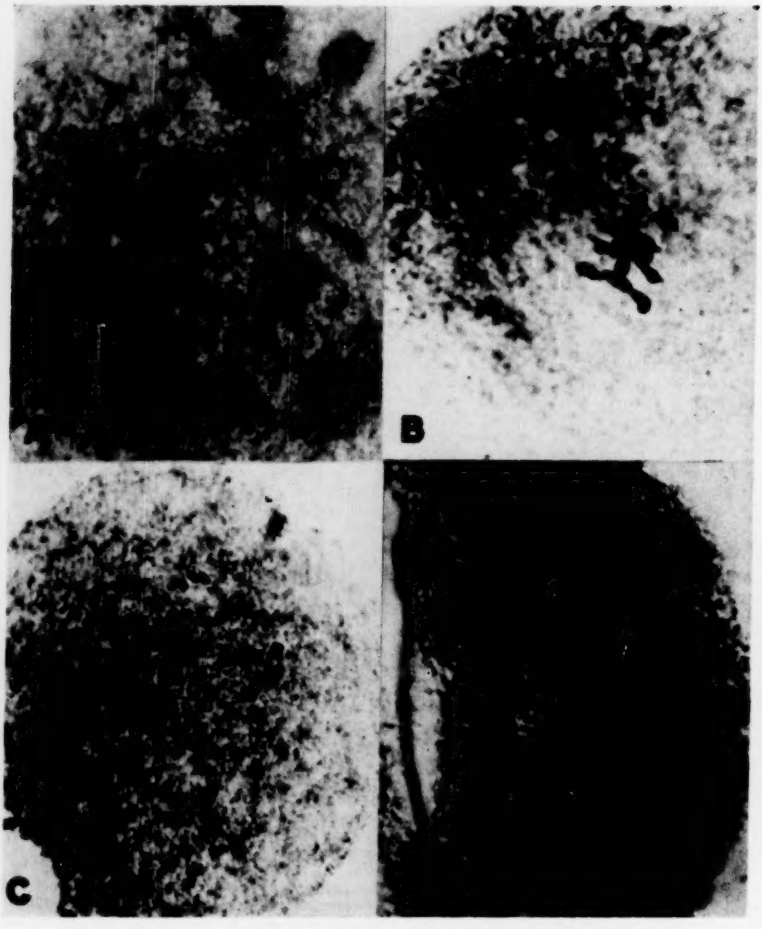
Although no investigations have been made of the cause of ovule abortion where the lethal ovule factor, lo_2 is present, studies of various stages of microsporogenesis have revealed that precocious desynapsis causes the meiotic irregularity which is responsible for the abortion of varying percentages of pollen grains.

The cells examined in the pachytene stage of prophase have shown no irregularity. Apparently all ten pairs of chromosomes are regularly and normally synapsed. However, at late diplotene it is apparent in some cells that instead of ten bivalents there are various combinations of bivalents and univalents. At diakinesis, where the chromosomes are still more contracted, this phenomenon of precocious separation can be noted in more cells and with a greater number of chromosome pairs (Figure 6*A*). One separated bivalent is found most frequently but 2, 3, 4, and 5 are also found. In the existing bivalents, most chiasmata appear to be terminalized at this stage. Many of the bivalents present are rod-shaped.

After desynapsis the univalents behave in much the same fashion as has been outlined by different workers in corn and other plants where a precocious separation has taken place. At metaphase the univalents are frequently found close to the equatorial plate although it is not unusual for one or more to be well removed from the plate (Figure 6*B*). At anaphase the univalents may act in one of two ways. The univalents which lie in the cytoplasm may be included in one of the daughter nuclei formed after the reduction division. The univalents on or near the equatorial plate lag behind after the bivalents have separated. They then divide equationally to give two chromosomes, one of which goes to each pole (Figure 6*C*). At the second meiotic division all of the chromatic material contained in each nucleus is oriented on the plate. The chromosomes which separated in a regular manner from

TABLE III. Backcross and F_2 progenies of crosses of partially pollen-sterile plants from *mn/mn* lines $\times$ *br/br* scored for pollen sterility, 1949 and 1950.

Pedigree	No. Plants with Normal Pollen	No. Plants with > 10% Empty Pollen	% Plants with > 10% Empty Pollen
(*br* × *mn* 1-7) × *mn* 1-7-2	149	68	31.3
mn 1-7-1-7 × (*mn* 1-7 × *br*)	34	18	34.6
mn 1-7-3 × (*br* × *mn* 1-7)	64	34	34.7
mn 1-7-2 × (*br* × *mn* 1-7)	51	15	32.6
(*mn* 1-7 × *br*) ☒	322	61	15.9
(*br* × *mn* 1-7) ☒	546	100	15.5
(*mn* 1-5 × *br*) ☒	274	45	14.1
(*br* × *mn* 1-5) ☒	261	65	19.9



MEIOSIS IN DESYNAPTIC PLANTS

Figure 6

A—Diakinesis with five pairs of univalents. *B*—Metaphase I with two pairs of univalents. *C*—Anaphase I resulting from precocious separation of four pairs of chromosomes. Note equational divisions in univalents lagging on equatorial plate. *D*—Telophase II illustrating lagging of chromosomes which underwent an equational division at Anaphase I.

their homologues at Anaphase I, will undergo an equational division, and the daughter chromosomes will move back to the poles. The same is true of univalents which have been included in one of the daughter nuclei after having been in the cytoplasm. But the chromosomes which are the products of desynapsis plus a division at the first meiotic division are unable to divide and are left on the plate (Figure 6*D*). They are then distributed at random to the quartet nuclei or excluded altogether and form micronulcei in the cytoplasm.

Table V gives the number of bivalents observed per cell for 403 pollen mother cells of

the partly pollen-sterile plant, 6505-7E1, examined at diakinesis. For chromosome 6 both homologues are attached to the nucleolus, and although separations can be noted, it is usually not possible to ascertain whether a given pair is associated as a rod bivalent as many of the other chromosome pairs can be seen to be or is completely separated. Therefore, chromosome 6 is always counted as a bivalent and the data represent the other nine chromosomes. Thus the average chance of a specific chromosome pair being present as univalents is 10.9 percent. On this basis a rough correction may be applied to the data to compensate for the omission of chromosome 6 (again in Table V). Beadle[2] has noted that the chances of obtaining a normal ten chromosome gamete from a cell with less than ten bivalents are $(\frac{1}{2})^n$ where n is equal to the number of unassociated pairs. In a calculation of this type using the corrected data from Table V, we find that 52 percent good pollen is expected. Actually the observed percentage of good pollen was 50.

The gene for precocious separation of the homologues with which we are dealing bears certain resemblances in its effects to *asynaptic*. There are important differences, however. This gene has no apparent effect on the meiotic process in the megaspores. Further, *as* is linked to *br* on chromosome 1 with 27 percent recombination (Beadle[2]). The F_2 data fail to show any linkage between *br* and this gene.

Because of the nature of the effect produced by this gene it is proposed that it be designated as *desynaptic* with the gene symbol being *dy*.

Discussion

Because of the generally accepted role of chiasmata in maintaining the bivalent condition after the pachytene stage of meiosis, it would seem that the origin of the univalent chromosomes might lie (a) in the failure of homologous chromosomes to associate properly, (b) in the non-formation of chiasmata after synapsis, or (c) in the unnaturally rapid terminalization of chiasmata after a period of normal association and chiasma formation. Pachytene preparations of this material show close synapsis between the homologues so that a lack of attraction between chromosome pairs cannot give rise to the observed univalents.

Beadle[2] in observations on *asynaptic* plants also noted that synapsis was normal but that the homologues frequently separated at pachytene without chiasma formation. Therefore, no crossing-over would occur in univalents. Where crossing-over was tested, it was found to be normal. This was attributed to the fact that a selected population of gametes (i.e. from pollen mother cells with ten bivalents) was available. Recently Rhoades[12] tested crossing-over between *sh* and *wx* in the diploid gametes of plants homozygous for *asynaptic* (by using the progeny of triploid individuals). Although such gametes would be produced in completely asynaptic plants and no crossing-over would be expected, Rhoades found that in a considerable number of the chromosomes tested there were cross-overs between the two loci. Apparently even in the asynaptic pairs there had been chiasmata formed but they had been resolved so rapidly that they played no part in keeping the homologues together after pachytene.

TABLE V. Distribution at diakinesis of the number of bivalents per cell for 6505-7E1 (50 percent pollen sterility).

Number of Bivalents	Number of Cells	Number of Cells (Corrected for Chrom. 6)
10	149	134
9	152	152
8	73	81
7	19	24
6	8	9
5	2	3

TABLE IV. The effectiveness of selection in partially pollen-sterile lines for progenies with high and low degrees of pollen abortion.

1947		1948		1949		
Plant	% Empty Pollen	Progeny	% Empty Pollen	Progeny	Range of % Empty Pollen	x % Empty Pollen
mn 1-2	25	*mn* 1-2-1	25	*mn* 1-2-1 ♂	35-50	45
		mn 1-2-2	50	*mn* 1-2-2 ♂	45-50	49
		mn 1-2-3	10	*mn* 1-2-3 ♂	10-50	25
		mn 1-2-6	15	*mn* 1-2-6 ♂	5-30	17
		mn 1-2-13	10	*mn* 1-2-13 ♂	5-50	15
		mn 1-2-16	10	*mn* 1-2-16 ♂	0-15	5
mn 1-7	40	*mn* 1-7-3	70	*mn* 1-7-3 ♂	20-100	65
		mn 1-7-7	65	*mn* 1-7-7 ♂	50-95	77
		mn 1-7-14	65	*mn* 1-7-14 ♂	20-85	50
		mn 1-7-15	35	*mn* 1-7-15 ♂	40-80	64

Moffett[9] in a study of chiasma behavior in *Anemone* observed that from diplotene to diakinesis there was a reduction in the number of chiasmata per bivalent and also a reduction in the number of terminal chiasmata per bivalent. This was interpreted as being due to the terminalization of the chiasmata followed by their resolution thus leaving the ends free. This behavior was explained as a loss of the "terminal affinity" postulated by Darlington.[7] Moffett pointed out that where there was both a strong tendency for terminalization and a loss of "terminal affinity" univalents would be expected but that crossing-over would have occurred.

For the gene with which we are dealing, a decision between the alternatives stated above does not seem possible in the absence of data of its effect on crossing over. This effect is now being studied. It is certain that for the diakinesis stage of prophase there is a reduced chiasma frequency in pollen mother cells of *desynaptic* plants as compared to the same stage in normal plants, but this would be expected in either case. It might be argued that whichever alternative (no chiasmata formed or precocious resolution of chiasmata) was correct, chromosome length would be expected to be critical in the sense that the shorter chromosomes would form univalents more readily. This does not appear to be so. In cells with nine bivalents the univalents were large chromosomes almost as frequently as small chromosomes.

It has already been stated that this gene affects the microspore mother cells, but has no effect on the megaspore mother cells. The inference was made since it was not possible to detect any degree of female sterility in plants homozygous for this gene, but it has been substantiated by the fact that there are no trisomics in the progeny of *desynaptic* plants. Desynapsis with formation of univalents and the possible random distribution at Metaphase I and II of these chromosomes is an effective mechanism for forming gametes with an extra chromosome. Pollen grains with an extra chromosome are not competitive with normal pollen grains, but the megaspores of such types give rise to trisomic plants. None could be detected in the progeny when *desynaptic* plants were used as female parents.

Summary

In a defective endosperm stock of maize which showed both pollen and ovule semi-sterility the ear semi-sterility is due to a lethal ovule factor which has no effect on microspore production and is transmitted only through the pollen. This gene is not lo_1 and is designated as *lethal ovule*$_2$, lo_2. The pollen semi-sterility has its origin in a desynaptic condition in meiotic prophase which is conditioned by a single recessive factor. Lacking genetic data on crossing-over it is not possible to determine whether the univalents arise as a result of the lack of chiasma formation or because of rapid terminalization of the chiasmata formed. The effects of the gene are not extended to megaspore formation. It is a different gene than Beadle's *asynaptic* and has been designated as *desynaptic*, *dy*.

Literature Cited

1. Beadle, G. W. *Genetics* 17:413-431. 1932.
2. ———. *Cytologia* 4:269-287. 1933.
3. ——— and Barbara McClintock. *Science* 68:433. 1928.
4. Burnham, C. R. *Genetics* 26:460-468. 1941.
5. Chittenden, R. J. and Caroline Pellew. *Nature* 119:10-11. 1927.
6. Clark, F. J. *Bull. Conn. Agr. Sta.* 465: 705-726. 1942.
7. Darlington, C. D. Recent Advances in Cytology. Philadelphia, Blakiston. 1937.
8. Lowe, Jeanette and O. E. Nelson. *Genetics* 31:525-533. 1946.
9. Moffett, A. A. *Cytologia* 4:26-37. 1932.
10. Pao, W. K. and W. H. Li. *Jour. Genet.* 48:297-310. 1948.
11. Rhoades, M. M. *Jour. Genet.* 27:71-93. 1933.
12. ———. *Genetics* 32:101. 1947.
13. Singleton, W. R. and P. C. Mangelsdorf. *Genetics* 25:366-390. 1940.

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VARIATIONS IN MITOSIS

II. The Interrelation of some Basic Deviations

G. B. Wilson, TeMay Tsou and Peter Hyypio*

IN a previous publication of this series[5] an attempt was made to investigate several variations in mitosis in terms of spindle activity. We were then especially concerned with "reductional" and "segregational" type divisions which appeared to us to lie somewhere between the extremes of "normal" dipolar arrangement and complete lack of organization as indicated by the formation of a single restitution nucleus leading to polyploidy. As pointed out by Huskins[2] such deviations have a considerable genetic potential since they can lead to reduction and segregation in somatic tissues. The present paper is concerned with a continuation of our efforts to find some logical order in the frequently observed variations in mitotic behavior. The hope is that such systematization, even though highly theoretical, may suggest more fruitful means of investigating the problem of chromosome mechanics than have been used in the past. It is our conviction that a sound arrangement of the morphological facts is a desirable and even necessary prerequisite to biochemical investigation and interpretation. We would not care to pretend that the scheme presented herein is more than a tentative first step, but one which we hope is in the right direction towards simplification and co-ordination.

Sources

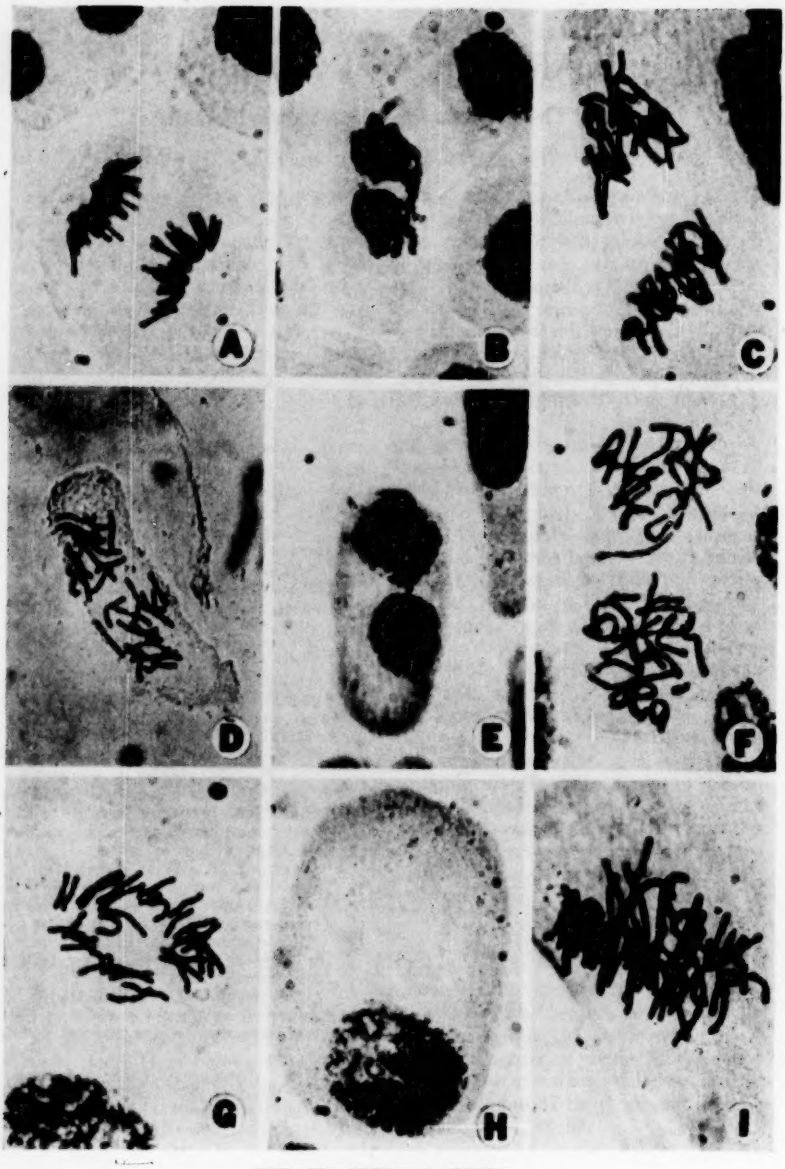
The majority of our observations and all of our illustrations have been taken from preparations of *Allium* root tips treated with either gamma-cyclochlorohexane or colchicine for various lengths of time ranging from 15 minutes to several days. As in the previous case, the treatments themselves have no special bearing on our immediate thesis and the illustrations could have been obtained from other sources including untreated tissue of one kind or another. In short, there is nothing new about the observations since similar configurations have been described and illustrated many times in the past by a great many workers.

Observations

Five deviations from standard anaphase behavior will be considered with respect to their possible interrelationships and consequences. These five are as described below:

1. *Failure of Cytokinesis:* A frequently observed immediate effect of cyclochlorohexane and, to a lesser extent, of colchicine is failure of cytokinesis at anaphase. After colchicine this failure is often and perhaps usually accompanied by cessation of anaphase movement. There is some indication of a similar correlation after treatment with cyclochlorohexane, but it is considerably less obvious. In any event the result is a binucleate cell in which the two nuclei are cytological and presumably genetical equivalents (Figure 7*A*).
2. *"Split" Anaphase:* Anaphase figures containing two or more separate spindles have been noted after a variety of treatments as well as in untreated tissue,[2,3] (Figure 8*A*). While it has been presumed that such anaphase configurations originate from "split" prophases this has, by no means, been proven to be the case. After treatment with either of the drugs used in the present instance, cytokinesis also normally fails. Assuming the eventual reinstitution of cell cleavage, the end result may be the production of cells containing a reduced number of chromosomes.
3. *"Star" Anaphase:* An anaphase organization which is superficially similar to the "split" anaphase, but which differs in some rather significant details, is very

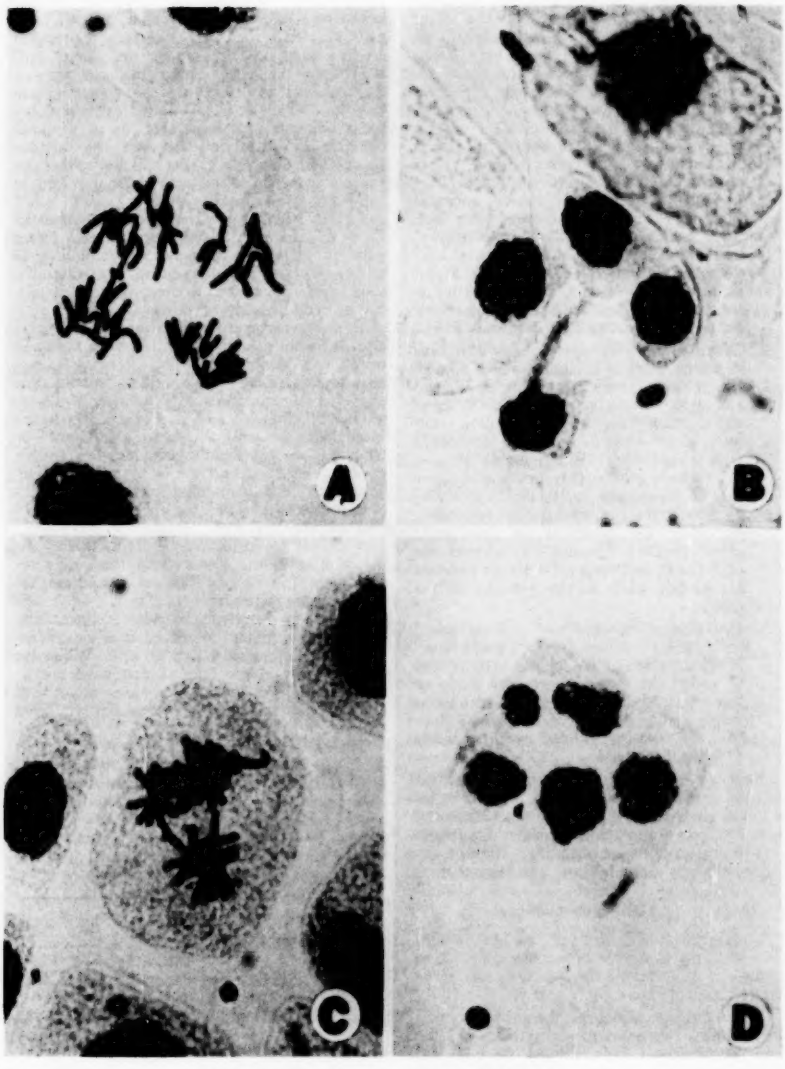
*Respectively Associate Professor of Botany and graduate assistants, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan. We wish to express our gratitude to the Dow Chemical Company, Midland, Michigan who supplied the cyclochlorohexane and to Mr. P. G. Coleman, Experiment Station Photographer, who prepared the plates. Contribution No. 52-16 of the Department of Botany and Plant Pathology, Michigan State College.



MITOTIC IRREGULARITIES

Figure 7

A—shows anaphase without cytokinesis; *B*—binucleate cell; *C*—double division figure with 16 chromosomes in each group; *D*—"segregational anaphase"; *E*—binucleate cells; *F*—double division figure with 14 and 18 chromosomes; *G*—"C-anaphase"; *H*—polyploid mononucleate cell; *I*—tetraploid metaphase. *A*, *C*, *D*, *F* and *I* are from cyclochlorohexane treated material, the rest are from colchicine treatments.



SPLIT TETRAD AND STAR ANAPHASE

Figure 8

A—shows "split" anaphase; *B*—"tetrad"; *C*—"star" anaphase; *D*—formation of microcytes. *D*—is from colchicine treated material, the rest are from cyclochlorohexane treatments.

common after short treatments with cyclochlorohexane and occasionally after similar treatments with colchicine. This configuration we have tentatively designated as "star" anaphase, the name deriving from the striking polar organization of the chromatids in which the kinetochores virtually fuse to form several centers of accumulation and the arms stick out in all directions without reference to the putative cleavage plane (Figure 8C). The fact that these configurations appear in large numbers after such brief treatments as 15 minutes indicate that they represent an immediate anaphase deviation rather than the result of an earlier cleavage. So long as treatment is continued these cells also show no sign of undergoing cytokinesis.

4. *"Segregation" Groupings:* Split figures in which there is no indication of spindle action have previously been designated as "segregational" groupings (see Wilson[5] and Figure 7D). They are fairly common after several hours of treatment with either drug. When initial division is into two groups they presumably give rise to binucleate cells, the two nuclei of which are not necessarily equivalent in either the genetical or cytological sense (Figure 7E and F). Again the evidence is that such cells do not undergo cytokinesis while in the presence of the drug.
5. *Unipolar or "C-anaphase":* The typical "c-anaphase" arises from "c-metaphase" in which there is no spindle activity and in which the chromosomes are more or less uniformly distributed. The result is a single restitution nucleus containing double the original chromosome number (Figure 7H).

Most of the commonly observed mitotic variants appear to fit into one or the other of the above categories or into a combination of them. We may, therefore, consider these variants as basic deviates including different degrees or kinds of spindle malfunction.

Discussion

An attempt to organize our five basic deviations in terms of degree of spindle malfunction as well as in terms of consequences has given rise to the chart shown in Table I.

One immediate effect of either drug used is the complete suppression of cytokinesis. So long as treatment is continued no cell will divide regardless of nuclear organization. In the simplest case (as shown in the top line of Table I) a binucleate cell results which is capable of undergoing a second mitosis which is normal if treatment is stopped. So far we have seen no indication that cytokinesis ever occurs in the initial cleavage plane.

"Split" anaphases and "segregational" groupings, though differing in degree of spindle breakdown, are allied in that they both logically may be assumed to arise from a previous separation in prophase or earlier. The "star" anaphase is not easily defined in terms of spindle malfunction. However, the appearance is as would be expected of mutual attraction between kinetochores separated by a minimal distance or attraction between the kinetochores and dispersed centers in the cytoplasm having properties similar to those ascribed to the poles of a normal spindle.

As indicated in columns two and three of Table I, the reinstitution of cytokinesis either with or without further mitosis may lead to a variety of end results not always distinguishable from each other. For example, both failure of cytokinesis in an otherwise normal anaphase and "segregational" groupings may give rise to binucleate cells which in turn result in cells with double division figures. Except where morphology of individual chromosomes allows distinction, the only method of determining whether a given double division figure came from a simple failure of cytokinesis or from a "segregational" grouping lies in the greater likelihood that the latter will result in binucleate cells containing different numbers of chromosomes in the two nuclei such as 18-14 (see Figure 7F).

A similar confusion may exist as to the origin of microcytes. Conceivably these may result from the reinstitution of cytokinesis in the immediate products of "split" or "star" anaphases or from multinucleate cells arising from "segregational" divisions involving separation into more than two groups. While we are currently of the opinion that most microcytes arise from "star" anaphases no critical evidence for this point of view has yet been obtained.

The more we consider the basic deviations described the more unlikely it becomes that the spindle can be adequately defined in terms of a single force. Although the characteristic metaphase-anaphase organization may be inhibited by a single drug, there appear to be several semi-separable zones of activity; namely, the zone of cytokinesis, the metaphase plate,

TABLE I
THE INTER-RELATION & CONSEQUENCES OF A SERIES OF MITOTIC VARIANTS

	I	II	III
NORMAL ANAPHASE	(Fig. 1A) "Failure of Cytokinesis"	(Fig. 1B) Binucleate Cell	(Fig. 1C) Double Division Figure 18/18
	(Fig. 2A) "Split" Anaphase	(Fig. 2B) Tetranucleate Cell	Chromosome Reduction
	(Fig. 2C) "Star" Anaphase	Multinucleate Cell	(Fig. 2D) Microcytes
	(Fig. 3) "Segregational" Grouping	(Fig. 4) Binucleate Cell	(Fig. 4) Double Division Figure (9-9/8-10 etc.)
	(Fig. 5) "C-anaphase"	(Fig. 6) Mononucleate Cell	(Fig. 7) Polyploid Cell
	NOTE: Stimulus Removed Between II & III		

NOTE: In the above Table Fig. 1 refers to Figure 7 and Fig. 2 refers to Fig 8. Figure numbers were changed after cut had been made.

the spindle "fibres" and the spindle "poles." On this view the spindle becomes an organizational complex which may exhibit various degrees of partial functioning between "normal" and complete elimination depending on the time and duration of action and kind of treatment applied.

While these various deviations may be considered atypical, they are not confined to treated tissue. For example, we have frequently noted binucleate cells in the tapetal layer of anthers especially in *Podophyllum*. Also split figures of various types are quite common in meristematic tissues of a variety of plants and complete failure of spindle activity is a common and in some cases a characteristic "accident" in a wide range of tissues of both plants and animals. The formation of microcytes occasionally follows aberrant second anaphase separation as shown especially in triploid and tetraploid *Musa*[4] and tetraploid spinach.[1]

Apart from the genetic potential inherent in the variations discussed, such deviations have a purely cytological significance in relation to the mechanism of mitosis. It is surely axiomatic that the manner in which a given mechanism misfunctions is inherent in the mechanism itself, and, therefore, a rigid analysis of what is involved in induced and spontaneous deviations as well as the conditions under which they occur should, in due course, provide definite limits to the choice of possible mitotic mechanisms.

Literature Cited

1. Bemis, W. and G. B. Wilson. (in press) 1952.

2. Huskins, C. L. *Jour. Hered.* 39:311-325. 1948.

3. ————and K. C. Cheng. *Jour. Hered.* 41:13-18. 1950.

4. Wilson, G. B. *Genetics* 31:241-258; 483-493. 1946.

5. Wilson, G. B., Mary Hawthorne and TeMay Tsou. *Jour. Hered.* 42:183-189. 1951.

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ORIGIN OF LIFE

THIS book is built of very large blocks of live contemporary biology made to cohere by a thick coating of an unusual compound—a philosophic amalgam of materialism ("critical naturalism"), purpose and teleology, God and Christianity; "the spurious concept of chance finds no place to insinuate itself." The origin of life, though outwardly granted to "natural law," is treated mainly as an area already exhibiting certain phenomena of importance to the book's argument. Sequence in organization leading to life is meagerly discussed; the coacervates and their literature are not mentioned; the full scale of viruses is viewed not as steps toward the living but as arrivals. The scant pages on the origin of life could have been written thirty or more years ago.

The "germ integer"—the molecule of chromatin, the gene—is the original living particle. In its modified, aggregated but concatenated forms it still is the builder of cells or of bodies (individuals), the receiver of impulses from cytoplasm or body that adaptively change it, and it is the vehicle of heredity and reproduction. The gene is of molecular nature; it is sustained by oxidation potential. Its structural changes—other than those involved in self-duplication perhaps—are purely adaptive changes made in purposeful response to environment when of evolutionary value. Neither natural selection nor fortuitous mutation plays a significant part in species formation. An argument for the effectiveness and directive influence of environment is elaborated. The view thus developed is believed to clarify both embryogenesis and evolution.

Most biological eyebrows can only lift at several affirmations of the preceding paragraph. Some readers of the book might concede that, if gene origins, modifications and activities followed the course prescribed, the facts of recapitulation and embryogenesis would be somewhat clarified. That concession, however, could only reinforce the need to examine the validity of the whole of the author's environmental theory of evolution. Within that broad scene each critic can select only one or two from among the many obtruding

The Origin of Life and the Evolution of Living Things: An Enviromental Theory. Olan R. Hyndman, M. D. XII + 648 pages. Philosophical Library, New York 1952. $8.75.

targets. In this review we first further quote:

"The (current) teaching is based on a theory that the species have evolved by random changes and that they exist because such changes were, by chance, not incongruous with the environment. My conclusions express a strictly opposite point of view. Living entities adapt *to* the environment—always tending to annul adverse influence". . . . Natural selection, "according to the theory proposed here plays only a small role in evolution. . . . Organic evolution and all change that it defines is the result and outcome of adaptation which is purposefully and directly controlled to maintain survival of the organismal type of species."

Perhaps it is here more appropriate and useful to present the author's views than to examine them. Comment is therefore restrained. However, in the author's view genes are the chief "living entities," and thus it is stated that genes "always tend to annul adverse influence." Do the numerous gene changes that have been observed following X-rays, ultra-violet, and chemicals confirm or deny? Moreover, in case a change in a gene means a change in a molecule, by what magic is the change in properties of this molecule restricted to a single one or kind? Again, the last of the statements quoted declares that all biological achievement is the "outcome of adaptation which is *purposefully* and *directly* controlled." Clearly, to Deity is assigned chief role in these affairs. Though "vitalism" is formally disavowed, "Let us assume that God is sufficiently omnipotent that His system is perfect in its beginning and throughout such that He does not have to interfere in order to grease the machinery, repair the cogs or play favorites."

"My thesis is a plea to reopen the case for Lamarck in respect to his conviction that environment determines adaptive change directly. . . . In respect to these fundamental concepts, the theory which I have formulated is in accord with the theory of Michurin (and Lysenko) and not in accord with the theory proposed by Weismann. . . . If mutation does not affect the quality of the genes, and this is the logical conclusion, then it is unable to account for adpative change except in a very limited sense. . . . Differences in living things are attributable to differences in environment at the cradle of life and thereafter."

One may acclaim the achievement involved in thus placing God and Lysenko on the same —and first—team. The reader of this long book will perhaps remain in doubt as to whether the nub of the treatise is science, philosophy, or a personal theology. Surely he will need to look widely for an attempt to blend philosophies more mixed and incongruous.

OSCAR RIDDLE
Plant City, Florida

HEREDITY IN UTERINE CANCER

THE purpose of this book,* which is published for the Commonwealth Fund, is to answer the question whether a disposition to cancer of a single site, uterine cervix, is inherited. Dr. Murphy is particularly well qualified to make such a study, having recently completed a modern study of congenital malformation of children in the Philadelphia area.

The book is divided into four section: I. The literature; II. Materials and methods; III. The results; IV. Discussion. The appendices give the cooperating groups, bureaus and hospitals, and the family record-form together with directions for completing it. The latter will be useful for the critical reader and for persons interested in further, or similar, investigations.

In the study, the occurrence of cancer among the relatives of 201 women who were suffering from cancer of the uterine cervix is compared to the findings for 215 control probands. 6445 individuals were included in the study.

Analysis of the statistics leads to the conclusion that genetic factors play a significant, though small, role in the etiology of cancer of the uterine cervix; that the factors which predispose to uterine cancer do not predispose in any significant degree to cancer in other sites; and that hereditary factors other than those concerned with uterine cancer, play a role in the etiology of cancer elsewhere in the body. The results have been obtained in spite of nearly insurmountable difficulties from a genetic point of view.

GEORGE W. WOOLLEY
Sloane-Kettering Institute
New York, N. Y.

*Murphy, Douglas P.: *Heredity in Uterine Cancer*. Harvard University Press, Cambridge. 1952. 128 pages. $2.50.

STUDIES ON THE MUTAGENICITY OF PHENOL

In *Drosophila melanogaster*

FRANK M. STURTEVANT, JR.*
Northwestern University, Evanston, Illinois

THE approach of chemical mutagenesis to the nature of the gene is much less drastic than that of radiation genetics. Those agents which have been found to be successful have been less effective, as a general rule, in producing high mutation rates and have even yielded "delayed effects."[2]

Although most of the chemicals which have been tested have been selected at random, this was not true of mustards or phenol. Mustard gas and nitrogen mustards were selected because of their penetration and extreme, radiomimetic reaction in living tissues. Niggli[15] chose phenol as a possible mutagen because it had been demonstrated to act as a Treffergift to *Botrytis* spores and *Escherichia coli*. Niggli believed that the mutagenic effect she demonstrated on *Drosophila* depended upon this Treffergift character. Moreover, she presented good evidence that there are certain loci whose chemical constitution and orientation make them more susceptible to phenol-mutation than other loci.

Mutagenicity has been demonstrated with phenol on *Antirrhinum majus*[16] and *Allium*,[13] but the only positive results on *Drosophila* were those of Niggli[15] using the technique of Hadorn.[5] In this method, excised larval ovaries are treated *in vitro* and then grafted to host larvae. All other attempts to produce mutations in *Drosophila* by phenol have met with failure: subjecting adult males to vapors or injecting them with the substance;[7] treating adult males by the aerosol method;[4] raising several generations on phenol-food or soaking fertilized eggs in phenol.[15]

The present report is on three new methods for treating *Drosophila* with phenol, with appropriate tests for locus-specificity.

Materials and Methods

The Oregon-R stock of *Drosophila melanogaster* was selected as a wild stock, and sex-linked lethals were determined by the Muller-5 method. For crossover estimates, Muller-5 heterozygotes were crossed to *yellow, hairy wing, white, miniature, forked* males. The standard error of crossover was determined from the variance of linkage estimate.[11] All cultures in all experiments were raised on the standard corn meal-agar medium in shell vials kept in a constant-light-and-temperature room at 22 ± 2°C.

The Vapor Experiments

Five half-pint milk bottles were arranged in series connected by glass and rubber tubing. The first bottle was attached to a compressed air inlet adjusted to deliver air at a rate of 4.0 ml/sec., as measured by water displacement. The air was first passed through mineral oil, then distilled water, saturated calcium chloride solution, phenol crystals (C.P., J. T. Baker Co.), and into the treatment bottle.

Experiment A1. Third instar male larvae of the wild stock were treated for 24 hours, and then removed to clean bottles where they were placed on filter paper moistened with Holtfreter's solution,[15] the normal saline used throughout all experiments. Only those larvae which had pupated during treatments were utilized, all others being discarded. After hatching, the males were mated individually to virgin, homozygous Muller-5 females. Heterozygous females of the F_1 were mated individually to three Muller-5 males, and the F_2 examined for the presence of lethals.

*From a dissertation submitted to the Graduate School, Northwestern University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Present address: G. D. Searle & Co., P. O. Box 5110, Chicago 80, Illinois. The writer is deeply grateful to Dr. George H. Mickey, under whom the present work was done, for his assistance and helpful criticism during the course of the investigation and in the preparation of the manuscript.

Experiment A2. For a confirmatory test of the effect of vapors on adult males,[6, 7] they were treated for 24 hours, then removed and mated as above.

Experiment A3. Even though females generally have a relatively lower spontaneous and experimental mutation rate than males,[10] they were tested also. After a 24-hour treatment, they were mated individually to three Muller-5 males. Virgin, heterozygous females of the F_1 were backcrossed singly, and the F_2 examined for the presence of lethals.

The Injection Experiments

A semimicro-syringe was devised which enabled the making of a small puncture in the larva, and whose delivery volume could be reasonably controlled. The syringe was made by attaching one end of a woven catheter to the needle of a one-ml tuberculin syringe, and affixing a small glass pipette to the other end. The hypodermic syringe was mounted on a heavy base, and the plunger worked by a hand screw. The delivery of the syringe per turn of the hand screw was calculated, and the optimal injection volume (0.012 ml) determined. Since up to one-half of that volume usually flowed out of the puncture when the needle was extracted, the volume remaining was estimated at 0.006 ml.

Late third instar larvae were inactivated by rolling them on filter paper with a brush; injection was accomplished by holding the larva under a dissecting binocular with a forceps and inserting the needle into the mid-lateral body-wall. The desired volume was administered, the needle removed, and the larva placed in a food vial. After hatching, the flies were tested by the Muller-5 method, as before.

All solutions were made with the same phenol as in the vapor experiments, using Holtfreter's saline (pH = 7.85) as solvent. The following injections were made: 25 male larvae with 0.20 per cent phenol (*Experiment B1*), 119 male larvae with 0.25 percent (*B2*), 122 male larvae with 0.50 percent (*B3*), and 47 female larvae with 0.50 percent (*B4*).

The Vaginal Douche Experiments

Following the method of Herskowitz,[8] mature sperm were treated with phenol by the vaginal douche technique. Virgin Muller-5 females, four to five days old, were given a vaginal douche of phenol solution with the semimicro-syringe described above. The fly was first etherized lightly, and then affixed on the stage of a dissecting binocular by moistening the wings. Extrusion of the ovipositor was effected by compressing the abdomen dorso-ventrally with a teasing needle. The glass pipette was then inserted, and phenol administered until it flowed out of the vagina. The wings were removed, and the treated females placed in an empty shell vial with four Oregon-R males which had been aged for a week.

Since Herskowitz[8] demonstrated by tests with dyes that substances did not enter the seminal receptacle and remained in the vagina for only about three hours, the treated females were left with the males only two hours. If mating did not occur during that period, those females were discarded. The remaining females were placed in individual creamers of food medium, and the resultant F_1 inbred singly. The F_2 was examined for the presence of recessive lethals.

Twenty females were treated with each of the following concentrations of phenol in Holtfreter's solution: 0.01 percent (*Experiment C1*), 0.10 percent (*C2*), 1.00 percent (*C3*), 2.00 percent (*C4*).

The Controls

It was necessary to establish two kinds of controls. The first (*Experiment D1*) was a special control for the injection experiments: larvae were treated exactly as for that series, except that saline only was injected. This control series was continued until it was established that the injection of saline did not significantly alter the mutation rate (Table I). Accordingly, 54 third instar male larvae were treated, and five tested by the Muller-5 method.

TABLE I. Summary of all Phenol Experiments and Comparison of Results to The Controls.

Exp.	Treatment	Phenol	Mortality	Sterility	No. P_1's	Chrs. Exam.	Lethals	Percent	Diff. /S.E.
A1	Vapors	24 hr.	—	25.0%	12	779	1	0.13 ± 0.13	0.7
A2	Vapors	24 hr.	—	0.0	16	812	1	0.12 ± 0.12	0.8
A3	Vapors	24 hr.	—	62.5	6	261	0	—	—
B1	Inj.	0.20%	80.0%	20.0	4	211	2	0.95 ± 0.67	1.0
B2	Inj.	0.25	77.2	11.5	23	1027	2	0.20 ± 0.14	0.3
B3	Inj.	0.50	86.9	0.0	14	736	0	—	—
B4	Inj.	0.50	78.7	0.0	8	190	0	—	—
C1	Douche	0.01	—	25.0	12	259	0	—	—
C2	Douche	0.10	—	37.5	10	213	0	—	—
C3	Douche	1.00	—	21.5	13	288	0	—	—
C4	Douche	2.00	—	50.0	1	19	0	—	—
D1	Saline	—	40.7	—	5	335	1	0.30 ± 0.30	0.2
D2	Untreated	—	—	—	32	1614	4	0.25 ± 0.12	—
D1 + D2					37	1949	5	0.26 ± 0.12	—

Thirty-two untreated adult males served as the main control (*Experiment D2*), that is, the spontaneous mutation rate was derived from Muller-5 tests on them. These control tests were carried on over the course of about two years, contemporaneously with the three main experimental series.

Results

In order to facilitate comparison of the results of the different experiments with each other and with the results of the control series, the essential data are gathered in Table I. Even though there is obviously no increase in mutation rate in the experimental series, the rates are compared statistically with those of the control series (*D1* + *D2*) in order to indicate the degree of significance of the data. The saline-injected controls (*D1*) are also compared to the untreated series (*D2*). The statistics in the last column of Table I are all below what is generally considered significant. Therefore, the differences between the experimental and control series can be explained by chance alone.

The average mortality in the *B* series was calculated at 80.7 percent, and, since the mortality in the control injections (*D1*) was 40.7 percent, the difference of 40 percent can be attributed to the injurious effect of the phenol injected in the *B* series.

The loci of all the lethals detected in the course of all experiments are listed in Table II; it is easily seen that they are distributed fairly evenly along the X-chromosome.

Although, in the data tabulated, only lethals were scored, it should be noted that at no time were any mutations detected which could be termed semilethal, i.e., the number of wild males obtained per Muller-5 F_2 culture was never below ten percent of that expected. The normal sex ratio had been calculated in a preliminary experiment to be 3,146 males to 3,318 females, or 1.00 : 1.05. Visible mutations were not considered, due to the personal equation.

All of the lethals, from the time of detection, were carried in stock by balancing them over Muller-5 chromosomes. At the time of writing, no penetrance of less than 100 percent has been observed, even though some of the lethals are now almost two years old.

Discussion and Summary

The lack of change of penetrance mentioned above does not agree with Hadorn's experiments,[6] in which the change may have been due to modifier accumulation or mutation of metastable premutations to stable energy levels (see Auerbach.[3])

The results from *Experiment A2*, in which adult males were treated with phenol vapors, confirm those of Hadorn, Rosin and Bertani[7] and Demerec,[4] who reported negative results. The present study indicates that other methods unsuitable for the demonstration of the mutagenic effect of phenol on *Drosophila* include subjecting male larvae and female imagos to vapors, injecting larvae of both sexes, and treating sperm by the vaginal douche technique. The only successful method yet to be employed is that of Hadorn,[5] in which ovaries are treated *in vitro*.

The reason for the failures is difficult to assess. Positive results have been obtained with the same techniques employed herein but with different chemicals. Thus, aerosols or vapors,[1] injections,[12, 14] and douches[8, 9] are all sound techniques, but, for some reason, are not effective for phenol work. It is noteworthy that all unsuccessful methods have involved administering phenol to whole organisms, while positive results were obtained only when the treatment was *in vitro*. The explanation seems probable that sublethal doses of phenol (1) are readily detoxified in the haemocoel of the larva before the mutagenic properties can become manifested, (2) do not reach the germ plasm when the latter is in a sensitive state, or (3) do not penetrate the membranes to reach the germ plasm at all. Obviously, the first case would not apply to the vaginal douche results.

It is usually assumed that maximal-injury dosages of chemical mutagens are most likely to be the more mutagenic. If gonadal injury is correlated with mutation rate, it is of interest to examine the lethal yield of such flies, even though the probability of discovering lethals in small numbers of progeny is admittedly lowered. By inspection, it was found that 29 of the experimental Muller-5 F_1 cultures contained 20 or fewer heterozygous females; no lethals were detected in any one of them. On the other hand, all six lethals coming from experimental series descended from F_1 cultures of 49 to 142 (average = 67) heterozygotes. Therefore, it may be concluded that the injury-mutagenicity hypothesis does not hold here, and that the reason the lethals were detected only in large F_1 cultures was because the probability of detection was greater in those cases. However, if younger stages in the development of germ cells are differentially sensitive to the

TABLE II. Loci of Lethals from all Experiments.

Locus ± S.E.	Experiment*
1.8 ± 0.78	*D2*
9.3 ± 2.56	*D2*
10.6 ± 5.39	*B2*
11.4 ± 4.45	*D2*
45.1 ± 0.83	*A1*
47.3 ± 3.59	*B1*
47.4 ± 3.29	*B1*
55.7 ± 3.71	*D1*
57.5 ± 3.12	*A2*
65.3 ± 4.20	*B2*
66.7 ± 2.67	*D2*

*No two lethals from the same F_1.

mutagenic action of phenol, as is known to be the case with formalin,[3] then mutations could be found only in the larger F_1 broods. Whether the lack of correlation between injury and mutagenicity would have held had the mutation rates been experimentally increased is a moot question, but in view of Niggli's data,[15] it seems improbable.

Literature Cited

1. Auerbach, C. *Biol. Rev.* 24:355-391. 1949.
2. ———. *Staz. Zool. Napoli* 22 suppl: 1-21. 1950.
3. ———. *Hereditas* 37:1-16. 1951.
4. Demerec, M. *et al. Carnegie Inst. Wash. Yrbk.* 45:143-157. 1946.
5. Hadorn, E. *Rev. Suisse Zool.* 53:486-494. 1946.
6. ———. *Staz. Zool. Napoli* 22 suppl: 32-49. 1950.
7. ———, S. Rosin and G. Bertani. *Proc. Eighth Int. Cong. Gen.*: 256-266. 1949.
8. Herskowitz, I. *Evolution* 1:111-112. 1947.
9. ———. *Proc. Soc. Exp. Biol. Med.* 70:601-607. 1949.
10. ———. *Science* 112: 302-303. 1950.
11. Hogben, L. An Introduction to Mathematical Genetics. W. W. Norton. 1946.
12. Law, L. *Proc. Nat. Acad. Sci.* 24:546-550. 1938.
13. Levan, A. and J. Tjio. *Hereditas* 34: 250-252, 453-484. 1948.
14. Lobashov, M. *Genetica* 19:200-241. 1937.
15. Niggli, H. *Genetica* 24:97-160. 1948.
16. Stubbe, H. *Biol. Zbl.* 60:113-129. 1940.

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Zebra Finch Mutations

INVARIABLY, when any species of wild-life is intensively bred by man, mutations are discovered and subsequently established. Interest in the exhibition qualities of the species then increases to the point that a new "Fancy" is born. Such has been the case with the humble Zebra Finch *Taeniopygia castanotis*.

For many years this species was looked upon merely as a rather free breeding species ideally suitable by temperament for a mixed aviary collection. As mutations occurred a few breeders turned to control breeding, using rather small breeding cages. They were highly successful both in their breeding operations, and in the establishment of the new types.

Today, we have not only the original wild type grey but also silver, cream, cinnamon, white, pied, white-headed grey, variegated grey, variegated fawn, variegated white, blue, black, silver-grey, buffs and pastel shades reported by various breeders as being established.

With these numerous mutant types in existence interest in the Zebra Finch as an exhibition species has boomed to the point that a Zebra Finch Society was organized in England last spring with Mr. Stan Moulson of 10 Eastern Avenue, Rose Hill, Oxford, England as its secretary. Since then this society has expanded to become an International organization with members in the United States, Canada, South Africa, Australia, New Zealand and numerous other countries.

In spite of the fact that numerous types are now well established, very little is known by members of the Zebra Finch Society concerning the nature and relationship of the various mutant factors involved. The society is therefore directing its first efforts toward locating such information as is now known concerning the genetics of the Zebra Finch. When the necessary information has been assembled, or determined through actual breeding tests, a series of tables of expectations will be compiled and made available to all Zebra Finch Fanciers.

Readers who have knowledge that would be of value to the Zebra Finch Society in this worthwhile effort are requested to correspond with either the Secretary, Mr. Stan Moulson, or with this writer.

Several members of the Society are fully qualified to make the required test matings, and are prepared to do so if this should be necessary. But, if the information now known concerning the genetics of the Zebra Finch can be assembled, it will be possible for the Society to make the desired information available to its members years sooner than would otherwise be possible.

Matthew F. Bender

P.O. Box 414
Mancelona, Michigan

TONGUE ROLLING AND TONGUE FOLDING

and other Hereditary Movements of the Tongue*

EDWARD E. GAHRES

STURTEVANT[4] first described a movement in which the lateral edges of the tongue were rolled together. He concluded that the ability was due to a pair of alleles. The gene conditioning the ability to roll the tongue (*R*) being dominant to the inability to roll the tongue (*r*). Urbanowski and Wilson[5] confirmed this conclusion. Hsu[2] described a pattern in the Chinese in which the distal part of the tongue folds back upon the base of the tongue. A population study revealed that this ability to fold back the tip of the tongue could only be expressed by those who had the ability to roll up the lateral edges. In other words, this represented a genic interaction—a phenomenon often recorded in other organisms but not prominant in man. Liu and Hsu,[3] in a later paper, postulated that a pair of alleles conditioned the tongue rolling and another pair of alleles was responsible for the tongue folding. The allele for rolling was dominant over its non-rolling allele and the folding allele was recessive to its non-folding allele. The data revealed that the folding-non-rolling class was absent, and that this was not due to chance. Liu and Hsu concluded that the two abilities are conditioned by complementary factors.

Another movement called "clover-leaf tongue" was first described by Hoch.[1] Whitney[6] also describes this movement and suggests that it may be a dominant trait with reduced penetrance.

This study is primarily a re-execution of Liu and Hsu's study in a Caucasion population. Other tongue movements were also investigated and will be discussed later.

Material and Methods

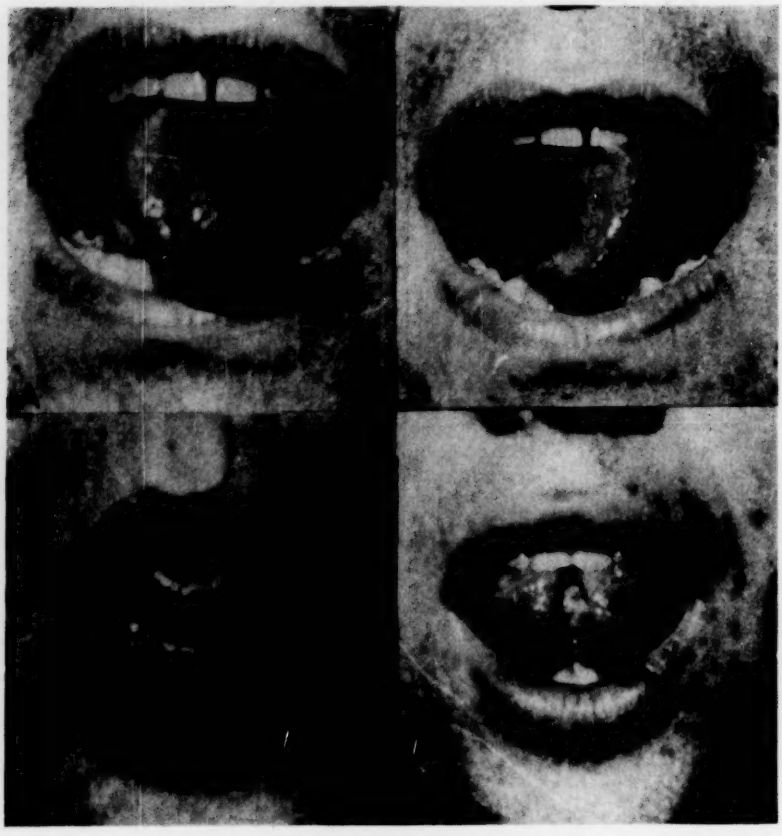
This investigation surveyed a sample of 865 residents of Eastern Coastal States. The data included grade school, high school and college students, and other adults, the preponderance of the data coming from students at the George Washington University and from the Washington, D. C., area in general. The population consisted of Caucasians of mixed European ancestory, for the most part. The psychological effect of discovering that one had what might be interpreted as a physical defect was absent in most cases. The difficulty of performing several of these movements probably added a greater source of error than the phychological effect. Some persons who might, with practice, form the various movements, were considered as being unable to perform the movement in question. These errors may have some effect upon the results, but it is believed that their effect is negligible as far as the conclusions here are concerned.

Results

Table I shows the total data obtained for the tongue rolling and tongue folding traits. The sexes are recorded separately. The symbol *r* represents the tongue rolling ability and *f* the folding ability. A plus sing indicates those persons who can perform the movement and a minus sign those who cannot. A greater percentage of females than males can both roll and fold the tongue. A chi-square test for random sampling revealed that the difference is non-significant and the sex difference may thus be attributed to chance. As in the data collected by Liu and Hsu, no persons were found who could fold but not roll the tongue. This absence is probably not due to chance, but is to be accounted for as Liu and Hsu suggested; by an epistatic interaction of the rolling and folding genes. The argument is as follows:

If *R-r* is a pair of genes responsible for tongue rolling, the genotype for people who can roll is either *RR* or *Rr*, and for those who cannot roll, it is *rr*. If *F-f* is another pair of genes responsible for tongue folding, the people who can fold have a genotype of *ff*, while those who cannot fold are genetically

*From a thesis submitted to the Faculty of the Columbian College of the George Washington University in partial fulfillment of the requirements for the degree of Master of Science.



TWISTERS AND CURLERS

Figure 9

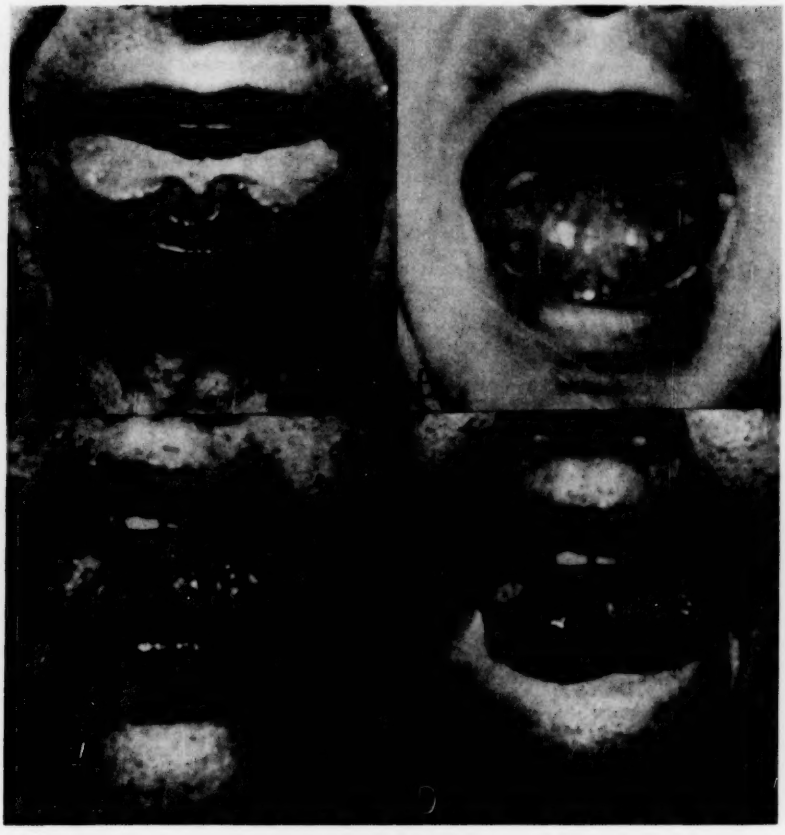
A and *B* illustrates the ability to twist the tongue first to the right and then to the left. Persons who can twist the tongue only one way are usually able, with practice, to twist it both ways. *C*—shows the lateral edge curling or rolling movement. *D*—shows a pattern in which the edges of the tongue are rolled up and flattened and then bent under, giving the appearance of having a split tongue.

FF or *Ff*. If there were no interaction between the genes *R* and *F*, we could expect four phenotypes to occur.

As was pointed out by Liu and Hsu, the two genes are not independent in their action. The folding ability can be expressed only by those who have the dominant rolling gene, *R*. Because of this interaction, the potential fold-non-rollers (*rrff*), can neither roll nor fold. Phenotypically, they are undistinguishable from the non-fold-non-roll class (*rrF*-).

Gene Frequency Analysis

According to the Hardy-Weinberg law, in a population mating at random, the frequency of the recessive allele of a pair of alleles is given as the square root of the frequency of the phenotype in the population. The allele frequencies, p and q of a pair of alleles in a random mating population in which three genotypes occur, express themselves in the proportions, $p^2+2pq+q^2=1$. Therefore, the gene frequency of the recessive allele is q,



CLOVER-LEAF AND FOLDING TRICKS
Figure 10

A—illustrates the clover-leaf pattern. *B*—illustrates the tip overfolding movement. This is accomplished by folding the tip of the tongue back upon the base of the tongue. *C*—shows the tip folding movement inside the mouth. *D*—shows the folding of the tip of the tongue outside the mouth. This subject "taught" the tongue to fold outside of the mouth by first extending the folded tongue.

and the frequency of the dominant allele is $(1\text{-}q)=p$.

In the case of R and r, the analysis is not complicated by this interaction. The results are shown in Tables II and III. In the case of genes F and f, the analysis is complicated by the epistatic interaction between the gene R and the gene F. In order to determine the proportions of the genotypically "fold-non-rollers," (*rrff*), it is necessary to take into account the phenotypic frequencies expected in a population containing two independently inherited pairs of alleles segregated at random. This relationship can be expressed in the following equation:

$$\frac{21\ (R\text{-}ff)}{616\ (R\text{-}F\text{-})} = \frac{x\ (rrff)}{228\text{-}x\ (rrF\text{-})}$$

This gives an expected number of 7.52 individuals who cannot perform the rolling and folding tricks contained among the *rrF*- group. This number is based on 637 individuals rather than the total number studied, but does not

impair the analysis. It is then necessary to correct the observed frequency and to put in the *f* category those non-folders who are homozygous for *f*, but who cannot fold because they lack the gene *R*. These corrected values are shown in Table II.

The frequencies of the alleles *r* and *f*, thus corrected, are, according to the Hardy-Weinberg law, $\sqrt{.2636}$ and $\sqrt{.0330}$ respectively. The gene frequencies of both pairs of alleles are shown in Table III.

Table IV shows the expected numbers of the three classes calculated from the above gene frequencies as compared to the observed numbers for the same classes. On the assumption that the *r-ff* class is absent by chance a chi-square value of 7.74 is obtained with one degree of freedom. The probability corresponding to this chi-square that the *rrff* class is missing because of chance is less than .01. If *R* and *F* are complementary, the expression of *f* must be dependent upon the presence of *R*. The agreement between the observed and expected frequencies on this hypothesis is almost perfect. This explains the absence of the double recessive phenotype in the population.

Other Tongue Movements

Table V indicates the number and percentage of people who can twist the tongue to the right only, to the left only or in either direction, as compared with those who cannot twist the tongue either way.

The results from the study of the clover-leaf pattern are shown in Table VI. Two types of clover-leafers were found: those who could immediately form the complete pattern, or could do so after a little practice; and those who demonstrated a limited ability to form a clover leaf pattern. Both these groups were tabulated together.

An interesting pattern was found in which the subject rolled up the edges of the tongue, flattened the rolled tongue, and bent it under giving the appearance of having a split tongue, (Figure 9*D*).

Discussion and Conclusion

From the data in Table IV, it can be concluded that the absence of the class which would be able to roll but not fold the tongue is not due to chance. If it is assumed that there is an interaction between the recessive allele conditioning folding and the dominant rolling allele, and that this interaction prevents the expression of the non-rollers-folders, the absence of this phenotype class is explained. This confirms the hypothesis of Liu and Hsu that there is an epistatic interaction between the rolling genes (*R-r*) and the folding genes (*F-f*). There does not seem to be any evidence of sex-linkage or sex-influence present in the action of either pair of genes. There does seem to be a slightly larger percentage of women than men who can fold the tongue.

From observations made in this study, the ability described by Whitney concerning the folding of the tongue inside and outside of the mouth may be due to tongue size and freedom of movement of the tongue rather than due to any special genetic effect. The ability to fold the tongue outside the mouth may be dependent upon other genetic mechanisms which are not in themselves in any way related to the folding ability.

In regards to the rolling ability, the data

TABLE I. Frequency of the rolling and folding traits.*

	r+ f+		*r+ f—*		*r— f—*		*Totals*	
Sex	No.	%	No.	%	No.	%	No.	%
♀	9	3.66	171	70.33	64	26.01	246	100.00
♂	12	1.94	443	71.57	164	26.49	619	100.00
Total	21	2.41	616	71.21	228	26.36	865	100.00

*A plus sign (+) indicates the ability, and a minus sign (—) the inability to perform the movement.

TABLE II. Proportions of the rolling and folding traits.

	- Rolling -		- Folding -	
Phenotype	+	—	+	—
Genotype	*RR-Rr*	*rr*	*ff*	*FF-Ff*
Actual No.	617	228	21.00	220.48
			(*R-ff*) 7.52	(*R-F-*)220.48
			28.52	816.48
%	73.64	26.36	3.30	96.70

TABLE III. Gene frequencies.

- Rolling -		- Folding -	
R	*r*	*F*	*f*
	.2636		.0330
0.487	0.513	0.919	0.182

TABLE IV. Expected and observed numbers of the three classes of tongue rollers and folders.

Genotype	*R-F-*	*R-ff*	*rrF-*	*rrff*
Phenotype	*r+ f+*	*r+ f+*	*r— f—*	*r— f+*
Observed	616	21	228	0
Expected	616	21	220.48	7.52

TABLE V. Twisting abilities in relation to sex.

	+		—		— Total —	
Sex	No.	%	No.	%	No.	%
♂	228	36.83	319	63.17	619	100.00
♀	91	36.99	155	63.01	246	100.00
Total	319	36.88	546	63.12	865	100.00

TABLE VI. Clover-leaf pattern in relation to sex.

	+		—		— Total —	
Sex	No.	%	No.	%	No.	%
♂	16	2.69	603	97.31	619	100.00
♀	6	2.44	240	97.56	246	100.00
Total	22	2.54	843	97.46	865	100.00

heretofore mentioned shows no sex difference in the ability to perform this trick. The percentage of people in the sample that can roll the tongue is almost 74 percent, which is slightly higher than has previously been reported.

No conclusions can be made as to the mode of inheritance of the twisting and clover-leaf patterns since pedigree data were lacking. The twisting ability is not dependent upon any other movement. The clover-leaf ability was found to be present only in those persons who could roll up the edges of the tongue. No one was found who could perform all four tricks. The clover-leaf pattern and the folding trick were not found to be present in the same person. This might be due to the fact that both abilities are present only in a small percentage of the population.

The gene frequencies for the rolling and folding abilities in this Caucasian population and the Chinese population are sufficiently different to assume that a racial difference exists between the distribution of these abilities in the two races.

The physical expression of the action of the rolling and folding genes probably involves the intrinsic muscles of the tongue. The extrinsic muscles probably initiate the tongue movements, but the patterns elicited are probably due to the intrinsic muscles. The length of the muscle fibers, and possibly the pattern of the intrinsic muscles seem to be the determining factors in each of the tongue patterns described.

Literature Cited

1. Hoch, Milton O. *Jour. Hered.* 40:132. 1949.
2. Hsu, T. C. *Jour. Hered.* 39:187-188. 1948.
3. Liu, T. T. and T. C. Hsu. *Jour. Hered.* 40:19-21. 1949.
4. Sturtevant, A. H. *Proc. Nat. Acad. Sci.* 26:100-102. 1940.
5. Urbanowski, Ann and Joan Wilson. *Jour. Hered.* 38:365-366. 1947.
6. Whitney, David D. *Jour. Hered.* 40:18. 1949.
7. ———. *Jour. Hered.* 41:176. 1950.

HAPLOID AND TRIPLOID SORGHUM

(*Continued from page 214*)

tially those of a good "diploid" species, although the supposed polyploidy of *S. vulgare* is indicated by the occasional bivalents in haploid cells as well as occasional tetravalents in diploid plants of this species (Chin[2]).

Literature Cited

1. Brown, Meta Suche. *Jour. Hered.* 34: 163-166. 1943.
2. Chin, T. C. *Am. Jour. Bot.* 33:611-614. 1946.
3. Gaines, E. F. and Hannah C. Aase. *Am. Jour. Bot.* 13:373-385. 1926.
4. Levan, Albert. *Hereditas* 27:243-252. 1941.

Harold J. Kidd

Oklahoma Agricultural Experiment Station Stillwater

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ELECTRON MICROGRAPH OF A *DROSOPHILA* CHROMOSOME

Figure 11

Electron micrograph of a portion of an unstretched salivary gland chromosome of *D. melanogaster*. Electron magnification of approximately 5,000× is enlarged to approximately 18,800×.

ELECTRON MICROSCOPY OF SALIVARY GLAND CHROMOSOMES

of *Drosophila*, using the Grid-Smear Technique*

IRWIN H. HERSKOWITZ

Department of Zoology, Indiana University, Bloomington

FOR almost two decades the chromosomes in the cells of the larval salivary glands of *Diptera* have been the subject of extensive investigation[7]. The giant size and characteristic cross-banded appearance have made these chromosomes a most valuable tool in the linkage of genetics to cytology and many of the cytogenetical advances of this period utilized maps of these chromosomes constructed from their readily observed gross morphology. There has not been, however, any universal agreement concerning their internal structure, and a number of hypotheses have been advanced to interpret the sub-microscopic morphology of these chromosomes from their appearance in the light microscope under various conditions. Recently it has been possible to employ the electron microscope for the examination of these chromosomes, thereby providing a much higher level of magnification for the study of their internal organization. Different techniques described for the preparation of salivary gland chromosomes for electron microscopy involve microdissection[1, 18], replica (cast) formation [14, 19], sectioning [15], and squashing (smearing).[17]

The purposes of this communication are to report a new and very simple technique for the preparation of salivary chromosomes for electron microscopy, and to present preliminary observations on electron micrographs of *Drosophila melanogaster* salivary chromosomes prepared by this method. The results obtained are compared with those of others using the electron and light microscopes, and their bearing on the interpretation of the fine structure of salivary chromosomes is discussed.

Methods and Results

Full-grown larvae of *D. melanogaster* are placed in 60 percent acetic acid, and the salivary glands removed. After 10 minutes in this solution the glands are transferred to a 200-mesh nickel grid previously coated with a water-floated film of parlodion. Another coated grid is put on top of this, the grids placed between two glass slides, and the glands crushed by thumb pressure. After the squashing, the two grids are separated with a needle and permitted to dry in air for a few minutes. The tissue is then ready for microscopy and remains in excellent condition for months.

Typical results obtained by this method, using an R. C. A. EMU electron microscope, are shown in the accompanying photographs. Figures 11-13 show regions of chromosomes which are relatively unstretched, and moderately and considerably stretched, respectively. Figure 14 shows, as do the others, a multitude of granules in the "band" and "interband" regions.

The grid-smear method is time-saving, permits microscopy of unstained tissues, and eliminates the necessity of subjecting the material to various chemical and physical agents, which might cause distortion in addition to the original fixation.

Discussion

It will be desirable to mention some of the variability of *Dipteran* salivary gland chromosomes in the living as well as fixed condition before discussing the contributions of electron microscopy toward an understanding of their structure.

*The experimental work was carried out at Louisiana State University School of Medicine where it was supported by a grant from the National Cancer Institute, U. S. Public Health Service. The electron microscope facilities were provided by the Charity Hospital of Louisiana and the Tulane University Biophysics Program

AN UNSTRETCHED CHROMOSOME

Figure 12

Electron micrograph of a portion of an unstretched salivary gland chromosome of *D. melanogaster*. Electron magnification of approximately 4,300× is enlarged to approximately 20,500×.

The nuclei in the salivary gland cells of living larvae may appear homogeneous (discounting the nucleolus), or as containing more or less banded chromosomes depending upon the species [2, 5], the amount of pressure applied to the larva, and other treatments.[3, 6] In living *Drosophila* larvae these nuclei appear homogeneous[9] or show sparse or faint banding.[2] Factors which affect chromosome thickness, stainability, and spreadability include the conditions in which the larva is cultured, the age of the larva, and the stage in maturity of the particular gland cells studied.

Salivary chromosomes fixed by different methods present various morphological appearances.[6] In *Drosophila*, for example, salivary nuclei retain the homogeneous appearance of the living state after fixation in 10 percent sucrose[16] or 4 percent neutral formaldehyde[8, 9] when the chromosomes almost fill the nucleus and show little or no banding either under ordinary or phase contrast microscopy. On the other hand, as is well known, fixation in acetic acid causes chromosomal shrinkage and the appearance of sharp banding.[11] In addition, as distinguished from formalin-fixed chromosomes, acetic acid fixation permits the chromosomes to be easily stretched lengthwise. Since the classic cytological picture of salivary chromosomes has been obtained from smears of acetic acid treated material it is worth noting that such chromosomes differ grossly in appearance, diameter, and length from normal living chromosomes. Nevertheless, the basic organization of the salivary chromosomes in the living state produces a pattern of banding of such reproducability, after acetic acid fixation and at the magnifications of the light microscope, as to be adequate for cytogenetic studies.

It seems apparent that any satisfactory interpretation of the basic structure of salivary chromosomes must take their physico-chemical nature into consideration. This must be done in studies of chromosomes in both the living and fixed conditions. In view of the difficulty of working with living salivaries most studies employ fixed material. In this case it is particularly desirable to take into account the physical and chemical changes produced by the fixation. Several chemical changes are known to be brought about by acetic acid fixation. Water is lost from the chromosome[6] and so is glycogen.[19] If a pellicle and matrix exist in the living salivary chromosome their chemical basis probably has been removed during fixation since electron micrographs have been unable to clearly demonstrate their presence. Certain physical properties may also be changed by acetic acid fixation. Since a clear solution of desoxyribonucleohistone, supplied by Dr. N. B. Kurnick, placed in acetic acid precipitated in sheets and fibers it is likely that this would happen to a similar solution contained in salivary chromosomes. From these considerations it remains important to determine to what extent differences between living chromosomes are represented by detectable variations in the cytological preparations, and to what degree the differences observed in the latter are due to the techniques of preparation.

It is almost universally accepted that the salivary gland chromosome is a multiple structure containing a number of reproductions of the basic chromosome material. According to the most widely accepted interpretation, the polytene hypothesis, the basic pattern is a thread, or chromonema, which reduplicates to produce a bundle of identical threads oriented parallel to each other.[13] However, some workers do not feel the evidence available is conclusive enough to identify the reduplicated material as a bundle of definite threads.[10] In any case, it is agreed by almost all workers that the basic unit of the mature salivary gland chromosome, whatever it may be, is below the limit of resolution of the light microscope.[8, 9] What evidence bearing on the fine structure of these chromosomes is obtained from the high magnification in electron micrographs?

It should be noted first that acetic acid fixation has been used in all the preparations of salivaries for electron microscopy so far reported and that the effects of such fixation on the induction of fibrils or on their appearance if already present in the living chromosome is as yet undetermined.

We know that the cross-bands seen in fixed preparations have a definite basis in bands which can be seen in the living chromosome. Accordingly, knowing the reality of these structures, study of their manifestations after various physical and chemical treatments can provide information concerning their properties in the living state. Studies on prepared chromosomes indicate some bands at least are multiple structures.[4] The largest number of bodies observed across the paired homologous chromosomes (appearing often as granules after acetic acid fixation) is about 30.[10] However, the number of units across these chromosomes is expected to be of the order of 1,000 when anlage and large salivary nuclei are compared as to volume[12] and as to polymerized desoxyribonucleic acid content[8, 9], assuming that the nuclear volumes and polymerized desoxyribonucleic acid content of these nuclei are reliable measures of the number of basic units present. It follows then that the score or so components of a band viewed in the light microscope are expected to be composed of a number of smaller units. This now has some cytological support in the electron micrographs of Pease and Baker[15], Yasuzumi, Odate and Ota[19] and the present work (see Figure 14, in particular). Although such observations are consistent with the polytene interpretation they

A MODERATELY STRETCHED CHROMOSOME

Figure 13

Electron micrograph of a portion of a moderately stretched salivary gland chromosome of *D. melanogaster*. Electron magnification of approximately 4,300× is enlarged to approximately 18,800×.

A BAND AND INTERBAND REGION

Figure 14

Electron micrograph of a "band" and "interband" region of a greatly stretched salivary gland chromosome of *D. melanogaster*. Electron magnification of approximately 12,200× is enlarged to approximately 44,500×.

do not favor it to the exclusion of a number of others.

Evidence concerning the nature of the lengthwise differentiation of the living salivary chromosome is more difficult to obtain. This is due primarily to the lack of any lengthwise orientation which can be seen in the intact living nucleus. However, strand-like connections between the components of one band and the next can be seen using micromanipulation on chromosomes isolated with an apparent minimum of distortion.[4] Pease and Baker were able to demonstrate in their electron micrographs numerous examples of fibers between small granules, distributed along the long axis, in chromosomes that were presumed unstretched during preparation. It is not apparent if these fibers are continuous between more than two such granules, nor clear why the photographs of relatively unstretched chromosomes by Palay and Claude[14] and the author (see Figure 11) do not show threads. On the other hand, stretched preparations of all workers distinctly show fibers (see Figures 13 and 14.) In view of the factors mentioned above it is concluded that electron microscopy has not yet provided support for any particular concept of the nature of the multiple structure of salivary gland chromosomes.

Summary

1. A grid-smear method of preparing *Drosophila* salivary gland chromosomes for electron microscopy is described and micrographs of chromosomes prepared in this manner are presented and discussed in relation to the work of others.

2. Photographs show that both the "band" and "interband" regions contain a large number of minute particles, providing further evidence of the multiple structure of these chromosomes.

Literature Cited

1. Ardenne, M. von. Elektronen-Übermikroskopie. *Berlin: Springer.* xvi + 393 pp. 1940.

2. Buck, J. B. (Abstr.) *Rec. Genet. Soc. Amer. 7*: 96. 1937; and *Genetics 24*: 96. 1938.

3. Buck, J. B., and R. D. Boche. *Collect. Net (Woods Hole) 13*:201-203; and (Abstr.) *Anat. Rec. 72 suppl.*: 73-74. 1938.

4. D'Angelo, E. G. *Annals N. Y. Acad. Sci. 50*: 910-919. 1950.

5. Doyle, W. L., and C. W. Metz. *Proc. Nat. Acad. Sci. 21*: 75-78. 1935.

6. ———. *Biol. Bull. 69*: 126-135. 1935.

7. Herskowitz, I. H. Bibliography on the Genetics of Drosophila II. Oxford, Alden Press. 1952.

8. Kurnick, N. B., and I. H. Herskowitz. (Abstr.) *Jour. Nat. Cancer Inst. 12*: 235-237. 1951.

9. ———. *Jour. Cell. Comp. Physiol. 39*:281-299. 1952.

10. Metz, C. W. *Cold Spr. Harb. Sympos. Quant. Biol. 9*: 23-39. 1941.

11. Metz, C. W., and E. H. Gay. *Proc. Nat. Acad. Sci. 20*: 617-621. 1934.

12. Muller, H. J. *Cold Spr. Harb. Sympos. Quant. Biol. 9*: 290-308. 1941.

13. Painter, T. S. *Cold Spr. Harb. Sympos. Quant. Biol. 9*: 47-54. 1941.

14. Palay, S. L., and A. Claude. *Jour. Exp. Med. 89*: 431-438. 1949.

15. Pease, D. C., and R. F. Baker. *Science 109*: 8-10, 22. 1949.

16. Ris, H., and A. E. Mirsky. *Jour. Gen. Physiol 23*: 489-502. 1949.

17. Schultz, J., R. C. MacDuffee, and T. F. Anderson. *Science 110*: 5-7. 1949.

18. Yasuzumi, G. *Cytologia* 14:1-9, 1947.

19. Yasuzumi, G., Z. Odate, and Y. Ota. *Cytologia 16*:233-242. 1951.

(*Received May 19, 1952*)

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Correction *in Re* the Tomato Genetics Cooperative and the American Tomato Yearbook

To the Editor:

I would like to call your attention to an error in the July-August (1952) issue of the JOURNAL. On page 172 under the caption "American Tomato Yearbook" information is included concerning two distinct publications. The American Tomato Yearbook is an independent publication not in any way sponsored by the Tomato Genetics Cooperative. The publication of the Cooperative is accurately described in the artcile, but it should be pointed out that the Report is a mimeographed one and available only to members of the Cooperative. The person to be contacted concerning membership in the Cooperative is not Dr. Carncross but is instead Dr. C. M. Rick, Division of Truck Crops, University of California, Davis, California.

It appears that in the article, the top paragraph of each column accurately describes the American Tomato Yearbook. The remainder of the material describes the Tomato Genetics Cooperative and its Report.

Donald W. Barton
N. Y. State Agri. Expt. Sta.
Geneva

CHROMOSOME NUMBERS IN COLEUS*

N. S. REDDY†

COLEUS consists of annual or perennial herbs widely distributed in Africa, Asia, and Australia. More than 200 species have been described; the actual number is certainly much less, for numerous varieties have undoubtedly been assigned specific rank (Rife and Dulser[9]).

Haberlandt[5], incidental to a study of the physiology of cell division, reported $2n=12$ and 16 for *Coleus*. Scheel[10] found $n=24$ for *C. rehneltianus* and $n=16$ for *C. aromaticus*. He suggested that he might have examined polyploid forms of the species which had been studied by Haberlandt. Furusato[3] published a $2n$-number of 24 for *C. blumei*. These data and results from the present study are summarized in Table I.

Four species of *Coleus* were studied during the present investigation: *C. blumei*, *C. hybridus*, *C. laciniatus*, and *C. forskohlii*. Seed of *C. hybridus* and of *C. laciniatus* were obtained from the Botanical Garden at Buitenzorg, Java; seed of *C. forskohlii* from the College of Agriculture, Coimbatore, India. Of numerous cultivated varieties commonly classified as *C. blumei* the following were studied:

"Golden Bedder"	Ohio State University
"Golden Bedder"	Purdue University
"Beckwith Gem"	Purdue University
"Max Levering"	Purdue University
"Elwood Kalin"	Purdue University
"Red Trailing Queen"	Purdue University
"Trailing Queen" (Type 1)	Ohio State University
"Trailing Queen" (Type 1)	Purdue University
"Trailing Queen" (Type 2)	Ohio State University

Aceto-orcein smears were used, without prior fixation of anthers, for study of microsporogenesis. Prefixation with either 1:3 acetic alcohol or Carnoy's fluid was found to result in weakly stained chromosomes. Aceto-carmine gave poor results. Propio-orcein generally stained prophase chromosomes better than aceto-orcein, but was not satisfactory for study of stages beyond Prophase I.

The procedure was to smear the four anthers of a flower in a drop of aceto-orcein, to remove the debris immediately, and with bees-wax and rosin to seal a cover slip over the preparation. The slide was not heated. After two to six hours the sealing compound was removed, the slide and cover slip pressed gently together between folds of blotting paper, and then resealed for study. Pressure applied in the conventional way immediately after the slide is prepared renders the preparation useless for study. When stored in a refrigerator the slides could be kept for two to three days. Preparations were made permanent by McClintock's schedule.[6]

Divisions in the pollen mother cells were most frequent on cloudy days. It was relatively easier to find meiosis in triploids, in "Trailing Queen" ($2n+1$), and in tetraploids than in diploids. Irregular meiotic divisions are seemingly of longer duration than regular ones. Triploids were the most irregular.

Somatic chromosomes were studied in two species. Warmke's method[11] was used on *C. hybridus* to make aceto-carmine smears of root-tips. Root-tips of *C. forskohlii* were fixed in Belling's modification of Navashin's fluid, embedded in paraffin, sectioned at 10μ, and stained with crystal violet.

An estimate of pollen viability was made from the percentage of pollen grains that stained fully in aceto-carmine. One plant exhibiting 95 percent viability in April showed only 40 percent in June.

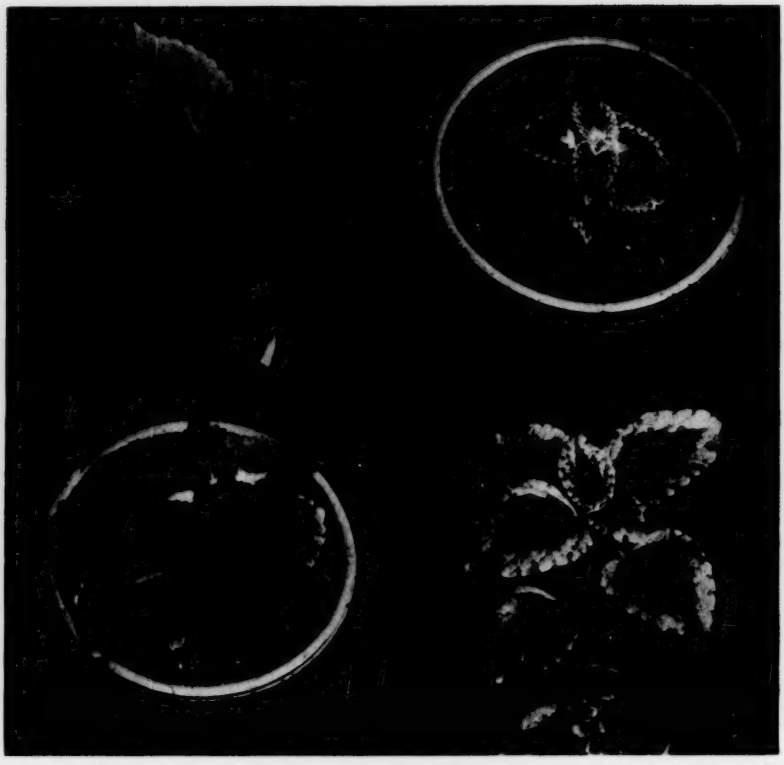
Size of guard cells was determined from epidermal peels. Length and width of 25 guard cells were measured in each case. The same peels were also used to determine the size of glandular-hairs. Length and width of 20 glandular-hairs were determined per plant.

The tetraploids used in this investigation were produced by soaking seed in 0.5 percent aqueous solution of colchicine for 24 hours.[8]

Photographs, taken at a magnification of 1000 times, are reproduced at about 1,100 times. Camera lucida drawings were made at a magnification of 3000 times and reduced one-half in reproduction unless otherwise stated.

*From a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at The Ohio State University, Department of Botany and Plant Pathology.

†Present address: Box No. 20, Rajahmundry, S. India.



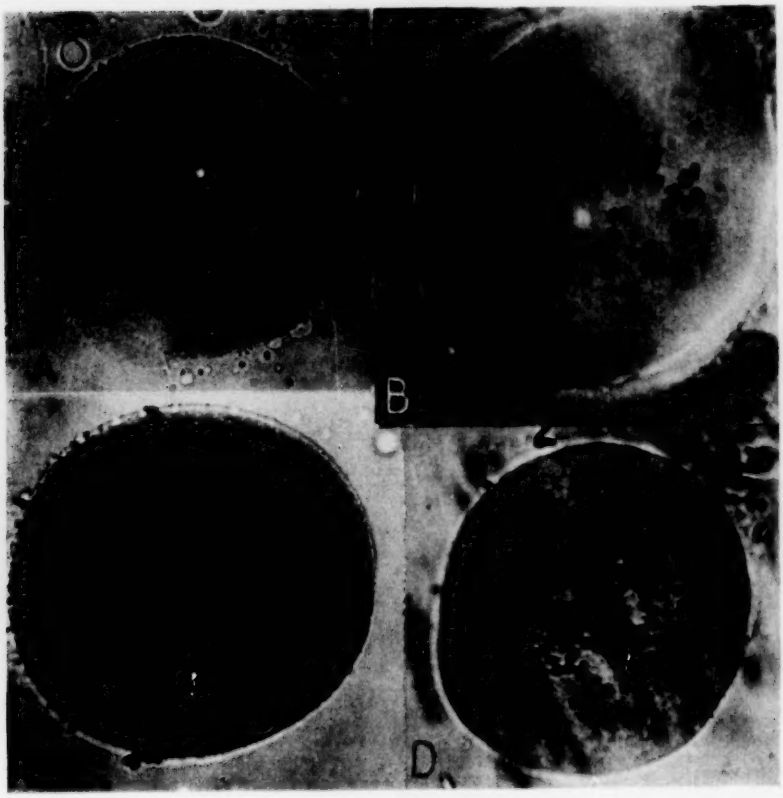
FOUR VARIETIES OF *COLEUS*

Figure 15

A shows a common cultivated *Coleus* of the Golden Bedder variety. *B* shows the Trailing Queen, clone 2. *C* is the triploid variety, Red Trailing Queen, characterized by 72 chromosomes. *D* shows the colchicine induced tetraploid variety, *C. blumei*. The chromosome configurations for the four varieties are shown in Figure 16.

Description of Species and Chromosome Numbers

Most cultivated varieties of *Coleus* are arbitrarily included in *C. blumei*. Bailey[1] is of the opinion that *C. blumei* represents an assemblage or combination of species, the horticultural forms being derived in part from *C. laciniatus*, *C. bicolor*, and possibly from *C. atropurpureus*. "Golden Bedder" (Figure 15*A*), characterized by solid yellowish-green color, is a typical example of this assemblage. Both clones studied had 24 bivalents at Metaphase II (Figure 16*A*). Three other varieties—"Beckwith Gem", "Max Levering." and "Elwood Kalin"—regularly had 24 chromosomes at Anaphase I. The exact systematic position of "Trailing Queen" (Figure 15*B*) is not clear; it is distinctly different from all other varieties. The plants are prostrate to semi-erect rather than erect; they are much branched, with leaves ¾ to 1½ inches long and about ¾ to 1 inch wide. The flowers are conspicuously larger than in many of the varieties assigned to *C. blumei*. Perhaps this variety rightly belongs under *C. rehneltianus*. Four different clones were studied. Three are alike except that one (Type 2) has an albinotic mid-rib. These clones have $n = 24 + 1$ (Figure 16*B*).



MEIOTIC DIVISIONS

Figure 16

A shows a cell of Golden Bedder during first meiotic metaphase. *B* is a microphotograph of a cell of Trailing Queen in anaphase taken from clone 2, showing a chromatin bridge. *C* shows a cell of Red Trailing Queen during late anaphase. *D* shows a cell of tetraploid *C. blumei* during early anaphase.

They have pollen fertility of approximately 35 percent and were not observed to set seed. The fourth clone, designated "Red Trailing Queen" (Figure 15*C*), is more vigorous than the other three. The leaves are larger, and the plant has red pigment throughout. The plant is triploid with $n = 36$ (Figure 16*C*). Pollen viability is 60 to 70 percent, but no seed have been set in the green-house.

Seedlings of *Coleus hybridus* vary greatly in leaf size, margin, and color. Most of these forms closely resemble the common cultivated varieties classed under *C. blumei*. Bailey and Bailey[2] consider that *C. hybridus* is a horticultural name for garden hybrids of unknown parentage. The three plants of this species cytologically examined had a $2n$-number of 48. The somatic chromosomes at metaphase vary in length from 2μ to 5μ.

Coleus laciniatus (Figure 17*A*) is characterized by an unusual type of venation: the veins anastomose in a more or less oval area. The leaves usually curl inward; leaf margins are more irregular than in the varieties of *C. blumei* (Rife and Duber[9]). Chromosome number at Metaphase I and Anaphase I is 24 (Figure 18).

Coleus forskohlii (Figure 17*B*) is commonly found growing wild in India and is not cultivated. It has a stout stem, woody at the

MUTANT LEAF SHAPE MIRRORED IN WILD SPECIES
Figure 17

A shows the peculiar leaf shape of *C. laciniatus*. This trait is due to a simple dominant gene. *B* is a wild species, *C. forskohlii*, found in India. Camera lucida drawings of the chromosomes of these two species are shown in Figure 18.

base and arising from a fleshy root-stock. Leaves are thick with short petioles. It has a $2n$-number of 28 (Figure 18). Though slight differences in size of chromosomes are evident, the range is less marked than in *C. hybridus*.

Progeny from Colchicine-Induced Tetraploids

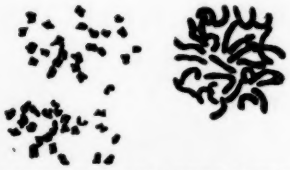
A gray plant (genotype PpG) of *C. blumei* was by colchicine treatment made tetraploid (Rife[8]). The tetraploid (Figure 15*D*) was poorly branched and slower in growth than the diploid. General development was much poorer in the tetraploid despite a greater cell size. It possessed a woodier and thicker stem. The leaves were slightly tougher but smaller. No increase in flower size was evident. Tetraploid nature (Figure 16*D*) of the plant was established by cytological examination (M. C. Green—unpublished). When selfed, the plant produced the following offspring:

Phenotype	Observed	Expected on the basis of chromatid segregation	
Purple	76	75.32	$\chi^2 = 0.0924$
Gray	110	108.36	D.F. = 2
Green	73	75.32	$P = 0.95$

Though data for the first generation could be explained on the basis of chromatid segregation, the progeny from crossing some of these plants with diploids showed anomalous segregation (Rife—unpublished). Plants of later generations had been classified as tetraploids purely on a morphological basis. Chromosomal study of certain of the progeny was accordingly undertaken. Seven individuals were investigated. One had a $2n$-number of 72; six had a $2n$ number of 48 and are therefore diploid.

The results indicate that the gray tetraploid may have produced all tetraploids in the first generation. These plants when crossed with diploids seemingly gave rise to maternal diploids: pollen of the diploids apparently served as an agent of stimulation. The single triploid is probably the fertilization product of a haploid gamete from the diploid and the diploid egg of the tetraploid. The diploids possibly resulted from haploid parthenogenesis. Numerous examples are known in which normal diploids have given rise to haploids and polyploids to polyhaploids. Such haploids and polyhaploids are usually sterile. There are a few reports of fertile diploids arising by parthenogenesis from tetraploids: Gairdner and Darlington[4] found a fertile diploid among the progeny of tetraploid *Campanula Persicifolia;* Randolph and Fischer[7] noted that selfed tetraploid maize regularly produced maternal diploids in the ratio of 1 diploid to 750 tetraploids.

Polyploid forms of *Coleus* could not be picked out with certainty on the basis of microscopic morphology. Degree of pollen fertility, stomatal size, and size of glandular hairs were therefore used to determine whether any one of these characters could be used for recognition of polyploidy. The tetraploid was compared with triploid "Red Trailing Queen" and with diploid "Golden Bedder" (Table II). Size of pollen, of guard cells, and of glandular hairs increased in positive correlation with number of chromosomes. Size differences in glandular-hair are conspicuous and could be used as an easy means of detecting polyploidy. Measurements can even be obtained on small plants, long before they flower.



***LACINIATUS* AND *FORSKOHLII* COMPARED**

Figure 18

On the left is shown a camera lucida drawing of chromosomes of *C. laciniatus*, taken from a cell during anaphase. The somatic chromosomes of *C. forskohlii* are shown on the right.

Summary

Cytological observations on four species of *Coleus* are presented. *C. forskohlii* has a $2n$-number of 28. The plant is not cultivated. *C. laciniatus*, a distinct species in cultivation, has an n-number of 24 and regular meiosis at microsporogenesis. *C. hybridus* and *C. blumei* each has a number of horticultural forms; the species are not readily separated. *C. hybridus* has a $2n$ number of 48. Three horticultural varieties of *C. blumei* have n-numbers of 24: one is trisomic ($2n = 48 + 1$); one is triploid ($n = 36$). A report of $2n = 24$ is in the literature for *C. blumei*. Two, perhaps three, additional species have been previously investigated.

A colchicine-produced tetraploid exhibited poor development and little vigor. When this plant was used as female parent in crosses with diploids, most of the progeny were diploid; one was triploid. Parthenogenesis is suggested to explain the production of diploids.

Literature Cited

1. Bailey, L. H. Standard Cyclopedia of Horticulture, Vol. II. The Macmillan Company, London, pp. 603-1200. 1914.

2. Bailey, L. H. and E. Z. Bailey. Hortus Second. The Macmillan Company, New York. pp. 1-788. 1941.

3. Furusato K. *Bot. and Zool.* 8: 1303-1311. 1940.

4. Gairdner, A. E. and C. D. Darlington. *Genetica* 13: 113-150. 1931.

5. Haberlandt. *Mitteilung Sitz. Ber. Akad. d. Wiss.* Berlin. 1919.

6. McClintock, B. *Stain Tech.* 4: 53-56. 1929.

7. Randolph, L. F. and H. E. Fischer. *Proc. Nat. Acad. Sci.* 25: 161-164. 1939.

8. Rife, D. C. *Jour. Hered.* 39: 85-91. 1948.

9. Rife, D. C. and H. C. Duber. *Jour. Hered.* 37: 327-330. 1946.

10. Scheel, V. M. *Botanisches Archiv.* 32: 148-208. 1931.

11. Warmke, H. E. *Stain Tech.* 10: 101-103. 1935.

TABLE I. Chromosome numbers in varieties and species of *Coleus*.

Species	Variety	Chromosomes		
		n	$2n$	Authority
Coleus blumei		—	24	Furusato (1940)
Coleus blumei	"Golden Bedder" Ohio State Univ.	24	—	Present report
Coleus blumei	"Golden Bedder" Purdue Univ.	24	—	Present report
Coleus blumei	"Beckwith Gem"	24	—	Present report
Coleus blumei	"Max Levering"	24	—	Present report
Coleus blumei	"Elwood Kalin"	24	—	Present report
Coleus blumei	"Trailing Queen"	24 + 1	—	Present report
Coleus blumei	Type 1			
Coleus blumei	Type 2			
Coleus blumei	Purdue Strain			
Coleus blumei	"Red Trailing Queen"	36	—	Present report
Coleus hybridus		—	48	Present report
Coleus laciniatus		24	—	Present report
Coleus forskohlii		—	28	Present report
Coleus aromaticus		16	—	Scheel (1931)
Coleus rehneltianus		24	—	Scheel (1931)
Coleus sp.		—	12-16	Haberlandt (1919)

TABLE II. Comparative cell size and pollen fertility at three levels of polyploidy in *Coleus*.

	Pollen fertility	Pollen Size	Size of guard cells		Size of glandular-hairs	
			Length	Width	Length	Width
	%	μ	μ	μ	μ	μ
Diploids (2n=48)	95.0	41.18 ± 2.01	24.58 ± 0.68	18.76 ± 0.86	48.96 ± 2.24	46.4 ± 1.76
Triploid (2n=72)	61.1	43.07 ± 1.63	29.32 ± 0.62	20.27 ± 0.73	55.36 ± 2.2	52.32 ± 1.19
Tetraploid (2n=96)	90.3	52.7 ± 1.53	39.64 ± 1.02	24.19 ± 0.85	68.32 ± 1.76	64.16 ± 1.63

(*Received August 7, 1951*)

HIGH AND LOW THYROIDAL RESPONSE TO THE FEEDING OF THIOURACIL

To New Hampshire Chickens

WILLIAM E. SHAKLEE and C. S. SHAFFNER*

IT is well known that the feeding of thiouracil† inhibits the normal production of thyroxine by the thyroid gland. The anterior pituitary then produces more thyrotrophic hormone which causes the thyroid gland to increase in weight. Considerable variation in the magnitude of the response to thiouracil has been encountered. Presumably, the individual variations in response are due to differences in the amount of thyrotrophic hormone released by the anterior pituitary gland, and/or a difference in the ability of the thyroid gland to respond to thyrotrophic hormone. If either of these functions is genetically controlled it should be possible to develop lines differing in their response to thiouracil.

A few workers have reported genetic differences in response of the chicken thyroid gland to goitrogens. Bates, *et al.*[2] obtained White Leghorn cockerels from two hatcheries and reported that they differed markedly in thyroid weight after treatment with thyrotrophic hormone. Mixner and Upp[6] fed thiouracil to baby chicks and found that the thyroids of double-cross hybrids from Rhode Island Red and White Leghorn inbred stock responded more than those from New Hampshire × Barred Plymouth Rock single-cross hybrids. Andrews[1] concluded from unpublished data that inbred lines of chickens differ widely in response of their thyroids to thiouracil. After one generation of selection, El-Ibiary and Shaffner[3] produced two groups of New Hampshire chicks whose thyroid glands differed significantly in weight when thioracil was fed.

Lines of chickens exhibiting differences in thyroidal activity would be valuable, not only to further the study of the inheritance of this characteristic, but also to furnish experimental material for the study of other characteristics known to be influenced by thyroidal activity. The lines would be useful in studying variations in rate of growth, feathering, reproduction, and other factors.

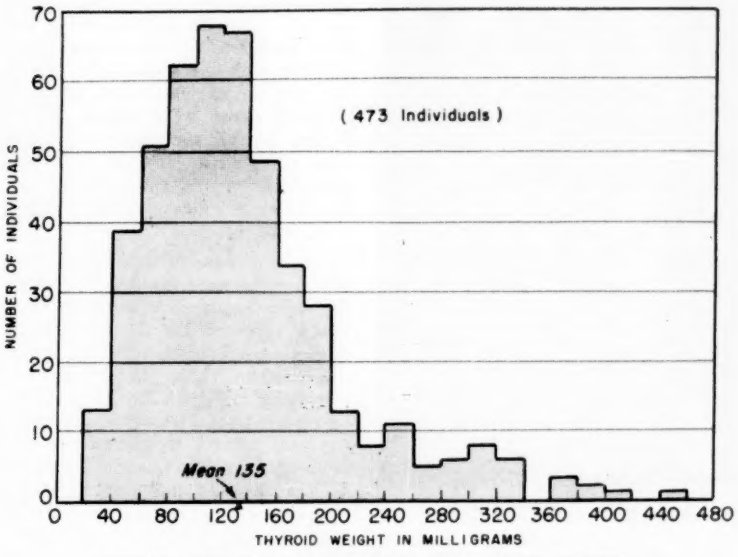
Materials and Methods

In June 1950 a plan was initiated to produce two lines of chickens, one with a high and one with a low thyroidal response to thiouracil. Chicks from various matings were fed thiouracil as 0.2 percent of the ration from the time of hatching until four weeks of age, at which time they were sacrificed. The thyroid glands were removed and weighed immediately with a Roller-Smith torsion balance to the nearest tenth of a milligram. Except for such natural selection factors as egg production, fertility, hatchability, and viability, the sole basis for selection was the average thyroid weights of full-sib families. Since it was necessary to sacrifice chicks to obtain thyroid weights, additional hatches were obtained for breeding purposes for the matings which produced extremes in thyroid weight. The families whose average thyroid weights were largest were designated the high line and the families whose thyroid weights were smallest were designated the low line.

Only 21 birds were available from the high-response and low-response groups described by El-Ibiary and Shaffner.[3] They consisted of four cockerels and seven pullets from the high-response group and four cockerels and six pullets from the low-response group. Matings within each line were made and a limited number of offspring was obtained. All offspring were raised for breeding purposes, none being used for determining thyroid response to thiouracil. Thus, one generation was produced without selection. However, because of the small number of birds available and the in-

*Poultry Department, University of Maryland, College Park. Scientific Paper No. A373. Contribution No. 2378 of the Maryland Agricultural Experiment Station.

†Thiouracil was furnished through the courtesy of Dr. Mark Welsh, Lederle Laboratories, Pearl River, N. Y.



THYROID WEIGHTS OF THIOURACIL-TREATED CHICKS

Figure 19

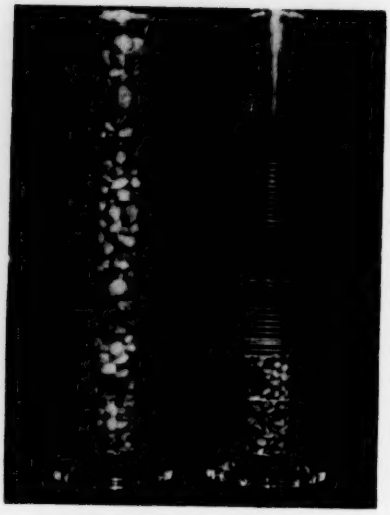
Frequency distribution of the thyroid gland weights of an unselected population. The chicks were fed a mash containing 0.2 percent thiouracil and were sacrificed when four weeks of age.

breeding which would have resulted from mating these individuals, it was decided to reinitiate the work and establish additional lines from the New Hampshire flock maintained at the University of Maryland.

Results and Discussion

In November 1950, six breeding pens were assigned to this project at the University of Maryland Poultry Farm and one sire and eight dams were placed in each pen. Three hatches of chicks were fed 0.2 percent thiouracil and at four weeks of age their thyroid glands averaged 135 mg. per bird. The frequency distribution for the thyroid weights of these unselected birds is shown in Figure 19. It will be noticed that the distribution is asymetrical or skewed, with the greater number of observations occuring at the left of the mean and toward the small-sized gland. This type of frequency distribution seems characteristic of thyroid gland weights of animals in general. Similar distributions were shown by Hollander and Riddle[5] in pigeons, by Mulligan and Francis[7] in dogs, and by El-Ibiary and Shaffner[4] in chickens.

Later in the hatching season diallelic matings were made. The sires in pens 8 and 9 were replaced by males which were the progeny of low-response birds, and the sires in pens 10 and 11 were replaced by males which were the progeny of high-response birds produced by El-Ibiary and Shaffner.[3] Table I shows the results of these matings. The average thyroid weight of each pen was higher in the second series than in the first series. The second series was produced from one to three months after the first, and it is suspected that environmental factors raised the levels of all pens in the second series. However, although no selection had been practiced on the generation in which they were hatched, the



HIGH AND LOW RESPONSE

Figure 20

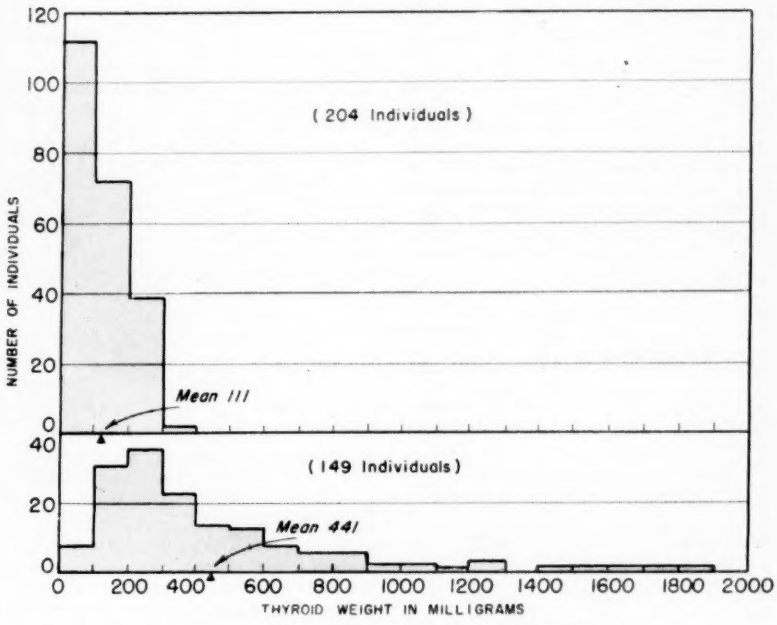
The thyroid glands from 100 chicks from high-response line (left) and 100 chicks from low-response line (right). All chicks were fed a ration containing 0.2 percent thiouracil and were sacrificed when four weeks of age.

high-response males produced progeny whose thyroid glands were much larger than glands from progeny of low-response males. The difference between the thyroid weights of progeny of the high-response and low-response males was highly significant, as shown in Table II. This increase was produced in spite of the fact that the high-response males were mated to females whose progeny by other sires averaged 21 mg. less than the progeny of females to which low-response males were mated. The difference between the coefficients of variation for the progeny of the high-response males and the progeny of the low-response males in Table I is highly significant ($P<.01$).

Selection was continued the following year by choosing families with highest thyroidal response for the high line and lowest response for the low line. Results obtained in the spring of 1952 are presented in Table III and are shown pictorially in Figure 20. After thiouracil treatment until four weeks of age, the average thyroid weight of the chicks from the high line was about four times as large as that of the chicks from the low line. The analysis of variance is shown in Table IV and the difference between the two lines is highly significant. The frequency distributions of the thyroid glands for the two lines are given in Figure 21. Each line exhibits the same type of skewed distribution as the original populations shown in Figure 19, but the variability is much greater in the high line than in the low line. The greater variability of the high line is also shown in Table III, the difference between the coefficients of variation for the two lines being highly significant.

TABLE I. Means and coefficients of variation with their standard errors of thyroid weights produced by thiouracil-fed progeny of diallelic matings involving unselected males vs. high-response and low-response males.

	First Series Progeny of unselected males			Second Series Progeny of low-response males		
Pen	No. of progeny	Thyroid wt. in mgs.	C.V. (%)	No. of progeny	Thyroid wt. in mgs.	C.V. (%)
8	100	148 ± 6	42.6 ± 3.0	105	166 ± 8	51.2 ± 3.5
9	59	156 ± 10	51.4 ± 4.7	70	180 ± 10	48.6 ± 4.1
Weighted means		151 ± 6	46.2 ± 2.6		171 ± 5	39.0 ± 2.1
	Progeny of unselected males			Progeny of high-response males		
Pen	No. of progeny	Thyroid wt. in mgs.	C.V. (%)	No. of progeny	Thyroid wt. in mgs.	C.V. (%)
10	101	120 ± 4	36.4 ± 2.6	107	236 ± 14	62.8 ± 4.3
11	60	148 ± 10	51.0 ± 4.7	54	169 ± 16	71.6 ± 6.9
Weighted means		130 ± 6	45.1 ± 2.5		213 ± 11	67.1 ± 3.7



AFTER TWO GENERATIONS OF SELECTION

Figure 21

Frequency distribution of the thyroid gland weights of the high-response and the low-response lines after two generations of selection. All chicks were fed a mash containing 0.2 percent thiouracil and were sacrificed when four weeks of age.

Summary

New Hampshire chicks were fed thiouracil as 0.2 percent of the ration from the time of hatching until they were four weeks of age, at which time they were sacrificed and their thyroid glands weighed. Full sibs of the families which exhibited the largest and the smallest thyroids, respectively, were raised for

TABLE II. Analysis of variance of thyroid weights produced by thiouracil-fed progeny of high-response and low-response males.

Source of variation	d.f.	Mean square	F
Between lines	1	1461	12.10*
Within lines	334	121	
Total	335		

*Significant at the 1 percent level.

TABLE IV. Analysis of variance of thyroid weights produced by thiouracil-fed chicks obtained after nearly two generations of selection.

Source of variation	d.f.	Mean square	F
Between hatches	1	203	
Between lines within hatches	2	45,518	94.36*
Within lines	342	482	
Total	345		

*Significant at the 1 percent level.

TABLE III. Means and coefficients of variation with their standard errors of thyroid weights produced by thiouracil-fed chicks obtained after nearly two generation of selection.

Low line				High line			
Pen	No. of progeny	Thyroid wt. in mgs.	C.V.(%)	Pen	No. of progeny	Thyroid wt. in mgs.	C.V.(%)
20	57	92 ± 6	48.3 ± 4.5	22	75	418 ± 36	70.3 ± 5.7
21	147	119 ± 5	51.8 ± 3.0	23	67	446 ± 46	81.8 ± 7.1
Weighted means		111 ± 4	52.6 ± 2.6			441 ± 28	75.9 ± 4.5

breeding purposes and were used to establish lines differing in their response to thiouracil.

The mean thyroid weight of the low lines has decreased very little but the thyroid weight of the high line has been increased to about four times that of the original population. This increase has been obtained after the equivalent of nearly two generations of selection and was accompanied by an increase in variability. The results reported here leave little doubt that the response of the thyroid gland to thiouracil is influenced greatly by heredity.

Literature Cited

1. Andrews, F. N. *Jour. Clin. Endocrinology.* 10:1142-1151. 1950.
2. Bates, R. W., O. Riddle and E. L. Lahr. *Endocrinology* 29:492-497. 1941.
3. El-Ibiary, H. M., and C. S. Shaffner. *Jour. Hered.* 41:246-248. 1950.
4. El-Ibiary, H. M., and C. S. Shaffner. *Poul. Sci.* 30:435-444. 1951.
5. Hollander, W. F., and O. Riddle. *Poul. Sci.* 25:20-27. 1946.
6. Mixner, J. P., and C. W. Upp. *Poul. Sci.* 26:389-395. 1947.
7. Mulligan, R. M., and K. C. Francis. *Anat. Rec.* 110:139-144. 1951.

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DISEASE RESISTANCE FROM A WHEAT × RYE CROSS

N. F. Jensen and G. C. Kent*

A SINGLE mildew and leaf rust resistant winter wheat plant was discovered in a progeny whose parentage traces to an original wheat × rye cross made by Dr. H. H. Love and the late Mr. W. T. Craig. The pedigree of this selection (Cornell Sel. 779b10-8-6) is Honor × Rosen rye; F_1 backcrossed to Honor; F_4 selection crossed to Yorkwin. None of the parents other than the original Rosen rye has shown any resistance to either wheat mildew or leaf rust.

Sel. 779b10-8-6 is wheat-like in all morphological respects except that it carries the "hairy-neck" marker which is characteristic of most rye plants. It resembles Yorkwin closely in appearance but has slightly weaker straw. It is fully self-fertile and has consistently excelled Yorkwin in yield trials. The white kernels are satisfactory in milling quality but harder than desired by some millers for a soft white wheat flour of top quality.

Since its discovery in 1942, Sel. 779b10-8-6 has been grown annually at Ithaca and elsewhere in New York State. Without exception the mature plants have been free of leaf rust even when grown adjacent to leaf rust susceptible varieties showing, in some cases, 100 percent infection. Dr. R. M. Caldwell found Sel. 779b10-8-6 to be highly resistant at Lafayette, Indiana, during a leaf rust epidemic in 1951.

The resistance of Sel. 779b10-8-6 appears to be a mature-plant resistance. Leaf rust rarely occurs in sufficient amount to make field readings possible on seedlings in the fall at Ithaca. Through the courtesy of Dr. C. O. Johnston, U.S.D.A. Pathologist, Manhattan, Kansas, however, seedlings of Sel. 779b-10-8-6 were tested with various races and found to be highly resistant to races 9 and 19, susceptible to races 5, 11, 15, 58, 93, 105 and 126, and segregating for susceptibility to races 21 and 45. These races were identified from collections made in New York during the period 1949-1951. Seedlings planted at Ithaca in late summer when rust is present have also shown only partial resistance or complete susceptibility so that it is quite clear that the resistance is the mature-plant type.

Transmission of leaf rust and mildew resistance in crosses to other wheats has been encouraging. Sel. 779b10-8-6 has been fully compatible in all crosses attempted. Both smooth and hairy-necked leaf rust resistant plants appear in the progenies of these crosses but in all cases noted, leaf rust resistance is associated with mildew resistance suggesting close linkage or pleiotropism of the genes involved. In nursery yield tests the best of the new hybrids, 82a1-2-4-7, a hairy-necked selection from a cross with Cornell 595 outyielded Cornell 595 by 23 percent in 1951 and by 3 percent in 1952.

Because of certain checks which can be made concerning the origin of Sel. 779b10-8-6, it is considered extremely unlikely that it is the result of a mixture or of a natural cross. The mildew and leaf rust resistance of Sel. 779b10-8-6 could have originated, through the transfer in the original hybrid, of one or more segments of rye chromatin by crossing-over or reciprocal translocation, followed by a novel recombination of genes in the final hybrid. The presence of the "hairy-neck" character is clear evidence that such transfer did in fact occur.

*Professors of Plant Breeding and Plant Pathology, Cornell University, Ithaca, N. Y., respectively. The authors wish to acknowledge the helpful suggestions of Professors L. F. Randolph and H. H. Smith.

C-MITOSIS IN COMMELINA COMMUNIS L.*

C. A. Berger, A. L. LaFleur and E. R. Witkus

Biological Laboratory, Fordham University

When root tips of *Commelia communis* L. were treated with colchicine to facilitate a chromosome count, interesting c-mitotic effects were noted. The chromosome number was determined from meiotic material as $2n = 90$ (Figure 22*A*). Root tips were treated for various periods of time with a 1 percent or a 0.1 percent solution of colchicine in tap water. After each treatment roots were fixed for both sections and smears. Material for sections was fixed in Craf fixative, sectioned at 10μ and stained with iron alum hematoxylin or the Feulgen stain. The material for smears was hydrolysed in equal part of 95 percent alcohol and concentrated hydrochloric acid for one minute. The tips were then immersed in a drop of aceto-orcein on a slide for 10 minutes. During that time the tissue of the root was teased apart. A cover slip was then placed over the tissue and slight pressure applied. Both the 1 percent and the 0.1 percent solutions of colchicine had the same general effect. However the 1 percent solution was better for showing the initial effects of colchicine, while the 0.1 percent was preferable for showing the recovery effects. Root tips were treated with the two solutions of colchicine for 2, 4, 6 and 8 hours. Some tips were studied immediately after treatment and others allowed to recover for 12, 24, 48 and 72 hours.

Results of Treatment with the 1 percent Solution

After two hours treatment with a 1 percent solution of colchicine three different c-mitotic conditions were noted. In many of the cells the c-chromosomes were distributed throughout the cell (Figure 22*B* and *C*). There were also many cells with clumped c-chromosomes (Figure 22*D*) and a few cells that showed the interesting type of exploded c-mitosis. No normal divisions were observed. In the exploded c-mitosis the chromosomes were forced to opposite sides of the cell. In the extreme type of exploded divisions the chromosomes were all located against the cell walls and no chromosomes were present in the center of the cell. In 1943 Barber and Callan[1] first described divisions of this type in newt mitosis following colchicine treatment and termed the phenomenon "exploded" mitosis. In the newt the cells did not recover from the exploded type of division. A similar type of division was termed "distributed mitosis" by Nybom and Knutsson[3] who observed them in the root tip cells of *Allium cepa* following treatment with methyl naphthoquinone. These exploded divisions were not as extreme as those reported by Barber and Callan[1]. Levan and Lofty[2] reported similar divisions in *Allium cepa* following colchicine treatment.

In our material after four and six hours treatment many of the cells in division showed the exploded or distributed type of c-mitosis (Figure 22*E* and *F*.) Many clumped metaphases were also present (Figure 22*G*). These clumped metaphases differed from those mentioned previously in that they were not centrally located in the cell, but occurred at one side of the cell. In some cases in *Commelina* the distribution of the chromosomes into two groups was approximately equal (Figures 22*H* and *I*, 23*J* and *K*). In other cells there was a great difference in the number of chromosomes at opposite sides of the cell (Figure 22*F*). Although the chromosomes separated in several groups, there was no evidence of segregation of homologous chromosomes into two different groups. The less extreme forms of the exploded divisions resemble normal anaphases at least superficially (Figure 22*H*). They differ from normal anaphases in the absence of a normal spindle and in the separation of whole chromosomes rather than chromatids.

In the preliminary work of this investigation it was determined that the 1 percent solution of colchicine was too strong to permit complete recovery of the root. Weaker concentra-

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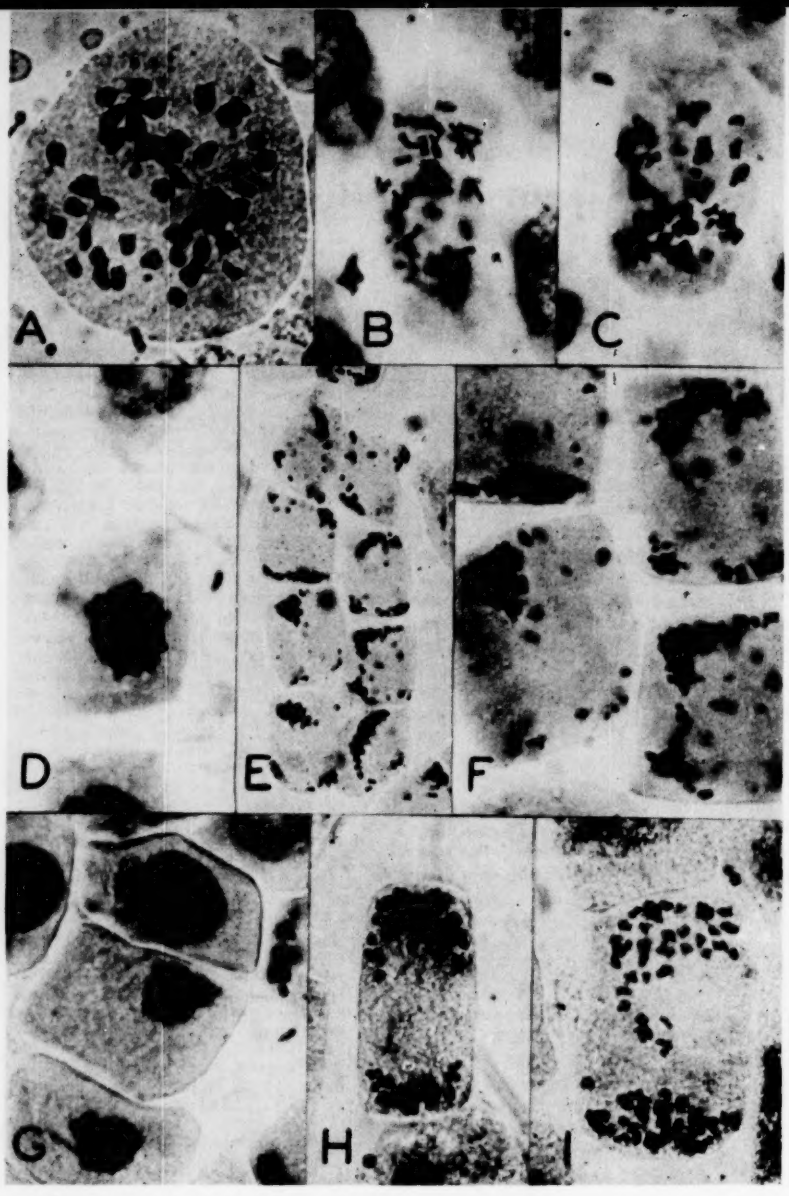
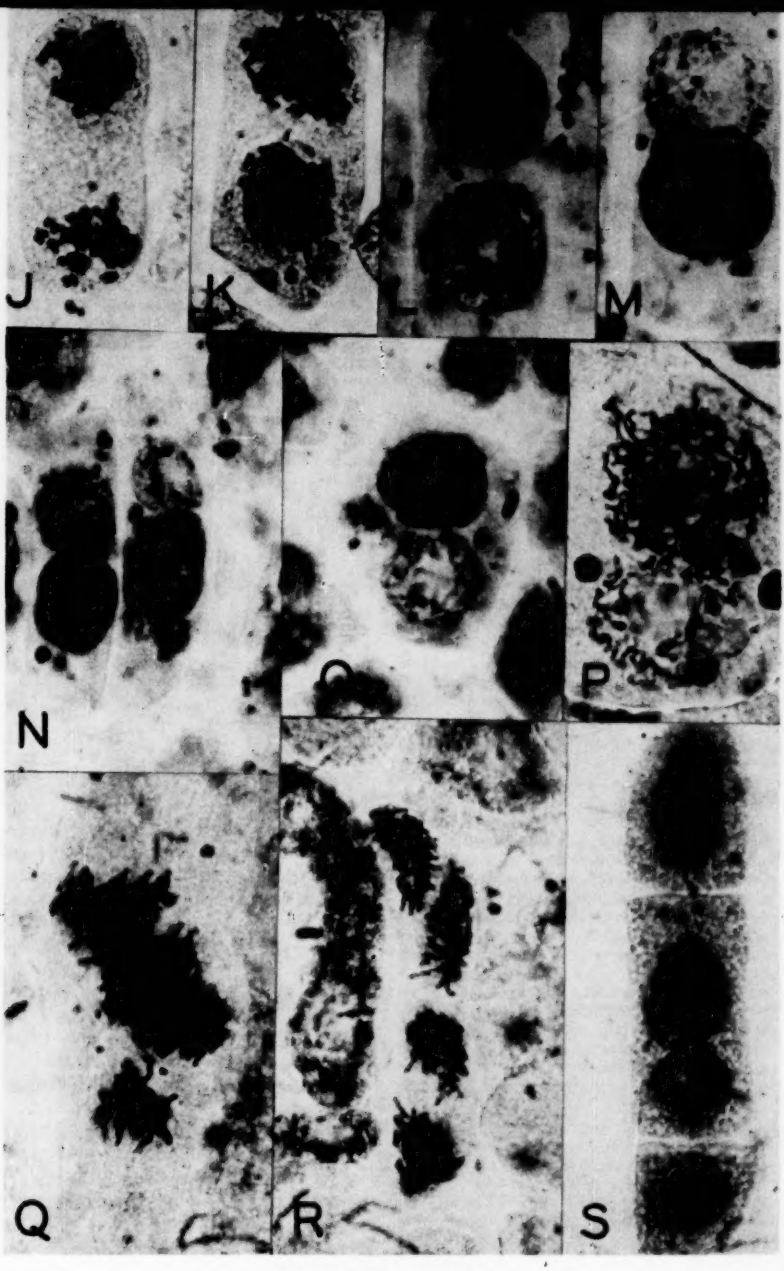


COLCHICINE INDUCED C-MITOSIS

Figure 22

Photomicrographs of aceto-orcein smear preparations of *Commelia communis* L. *A*—Diakinesis showing the 45 bivalents. *B*-*C*—Cells with c-chromosomes distributed throughout the cell. *D*—A cell showing a clumped c-metaphase. The chromosomes are centrally located. *E*—Meristematic cells all showing the extreme type of exploded c-mitosis. *F*—Meristematic cells showing the extreme type of exploded c-mitosis. *G*—A cell showing a clumped c-metaphase. The clump is peripherally rather than centrally located. *H*—A less extreme form of the exploded division. The chromosomes are segregated in two almost equal groups. *I*—Incompletely exploded c-metaphase. Several chromosomes can be seen in the center of the cell. All magnifications are 1,010×, except *E*, which is 402×.



EXPLODED C-MITOSIS

Figure 23

J—Another example of a moderate form of an exploded c-metaphase. *K*—A stage in the reversion of the c-anaphase chromosomes to the resting stage. Both nuclei have about the same number of chromosomes. *L-N*—Binucleate cells showing the diverse staining reaction and micronuclei scattered throughout the cytoplasm. *O*—A binucleate cell in early prophase. The prophase chromosomes in the two nuclei show a different staining reaction. *P*—A binucleate cell in later prophase. *Q*—A binucleate cell in metaphase. Note the difference in the size of the metaphase plates. *R*—A binucleate cell in anaphase. *S*—Three cells resulting from the previous division. The central cell is binucleate. The end cells are uninucleate. All magnifications are 1,010×.

tions were then used in the later experiments. The initial effects shown by the different concentrations of colchicine were similar. It was found that the 0.1 percent solution gave the same initial effects as a 1 percent solution and still permitted complete recovery of the root. The recovery effects of the 0.1 percent solution will be described in detail.

Recovery Effects Following Treatment

A 0.1 percent solution used for eight hours showed very good initial effects as well as recovery effects. After eight hours treatment with a 0.1 percent solution no normal metaphases or anaphases were found. Most of the cells showed the exploded type of c-mitosis. In many cases the separation of the chromosomes into two groups was complete. In other cases there was only a partial separation of the chromosomes into two groups and c-chromosomes could be seen between the two groups (Figure *22I*). After twelve hours recovery following this treatment, the SA-regions of the chromosomes divided and the chromatids separated slightly. This stage has been termed distributed c-anaphase by Levan and Lofty[2]. After the division of the SA-regions, the chromosomes undergo reversion to the resting stage, still maintaining their original grouping (Figure *23J* and *K*). Frequently the two nuclei in the same cell reacted differently to the stain used, one nucleus staining much more faintly than the other (Figure *23L*, *M* and *N*). This was true in the case of aceto-orcein smears and of the iron alum hematoxylin and Feulgen stained sections. In some cases the lighter staining nucleus was the smaller of the two nuclei, in other cases it was the larger. Frequently they were of a similar size. This probably indicates a difference in the amount of nucleic acid in the two nuclei which difference is not correlated with the size of the nucleus. One or more nucleoli may be present in each nucleus. The difference in the staining reaction of the two nuclei could not be correlated with the number of nucleoli.

This type of division cannot be considered a reduction division, since both nuclei are in the same cell and there is no cell plate formed between them. Nor can it properly be called somatic reduction, since it does not involve the separation or segregation of homologous chromosomes. After 24 hours recovery, both nuclei in the cell divided simultaneously (Figure *23O-S*). Micronuclei are frequently present in these dividing cells (Figure *23P*). The difference in the staining reaction of the two nuclei is also evident in the prophase of this division (Figure *23P*). At metaphase there are two spindles formed in the same cell. These spindles and the accompanying metaphase plates may be of the same or unequal size, depending on the original separation during the exploded c-mitosis (Figure *23Q* and *R*). The spindle attachment regions of the chromosomes divide and the sister chromatids undergo normal anaphase separation (Figure *23R*). During telophase cell plates are formed between two groups of sister chromatids. There is no cell plate formed, however, between the two original nuclei of the binucleate cell. As a result of this division three cells are formed. One, the central cell is binucleate. The other cells, the extreme ones, are uninucleate (Figure *23S*). The number of chromosomes present in these nuclei is dependent on the separation at the time of the exploded c-mitosis.

Discussion

Both Barber and Callan[1] and Nybom and Knutsson[3] have attempted to explain the origin of the exploded type of c-mitosis. Barber and Callan have suggested the presence of a partial spindle, the centrosomal spindle, as the causal mechanism. Nybom and Knutsson assume the presence of a spindle tactoid, the particles of which push the chromosomes in a peripheral direction.

In plants there is no centrosomal spindle so the explanation of Barber and Callan cannot apply to exploded c-mitosis in plant material. The tactoid particle explanation is still purely hypothetical. The evidence from *Commelina* indicates that the central region of the protoplast undergoes great physical modification, in sectioned material appearing as a swelling vacuole, which forces the chromosomes centrifugally to a very unusual position against the cell walls.

The more normal type of c-mitosis, as it appears in *Allium* and most other plants, is an efficient means of producing eupolyploid cells. The exploded type of c-mitosis is most inefficient in this regard, giving rise to a great variety of aneuploid multinuclei. The high frequency of exploded c-mitoses in *Commelina* and their relative infrequency in *Allium* and other plants indicates a unique difference in the protoplasmic structure of *Commelina*.

Summary

1. The chromosome number of *Commelina communis* L. was found to be $2n = 90$.
2. A 1 percent and 0.1 percent solution of colchicine after four hours resulted in an exploded type of c-mitosis in *Commelina*.
3. The distribution of the chromosomes was unequal in most cases.
4. The exploded type of c-mitosis resulted in binucleate and multinucleate cells.
5. Frequently the two nuclei in a binucleate cell differed in their staining reaction.
6. Upon recovery, binucleate cells underwent mitosis giving rise to three cells, of which the central cell was binucleate.
7. The distribution of chromosomes in an exploded c-mitosis is at random and most of the resulting combinations are aneuploid.

Literature Cited

1. Barber, H. N., and H. G. Callan. *Proc. R. Soc. B*, 131:258-271. 1943.
2. Levan, A., and T. Lotfy. *Hereditas* 35:337-374. 1949.
3. Nybom, N., and B. Knutsson. *Hereditas* 33:220-234. 1947.

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FRONTAL HAIR DIRECTION

In American Chinese, Indian and Negro Populations

THE inheritance of the different types of frontal hair directions in man has been previously discussed in this Journal[1,2]. The interpretation that the different types may be a result of a competition between two growth-inducing centers of the skin has been confirmed by experiments on rats[3]. Thus, if the skin growth from the nasal angles of the eyes prevails, this gives the strongest dominant Type III. If the growth from the center divergence in the "crown" prevails, this will produce the weakest recessive Type I. Intermediate types, defined by special limits are designated Type II and are recessive to Type III, but dominant to Type I.

An investigation of the frequencies of these different types in mentally deficient individuals showed interesting differences as to the geographic distribution, indicating higher frequency of Type III in Africa and Southern Europe, while Types I and II seem more frequent in Eastern Europe[2].

In order to get a more representative group to elucidate this problem, examinations of three different American populations of predominantly non-European descent were made by the author in 1948*.

The material consists of 348 American Chinese children all pupils of the public schools of San Fransisco; 304 young American Indian's from the Albuquerque Indian School; and 160 Negro patients (mostly children) from the Charity Hospital, New Orleans.

Practically the entire Chinese population of San Francisco originates from Canton and Hong Kong in South China and a number of the children examined were themselves emi-

TABLE I. Frequencies of the major types (I, II, III) of frontal hair direction in three different American populations.

Population	I		II		III		Total
	n	%	n	%	n	%	
American Chinese:							
Male	185	88.5	15	7.1	9	4.3	209
Female	129	92.8	1	0.7	9	6.5	139
Total:	314	90.2	16	4.6	18	5.2	348
American Indians:							
Male	160	80.8	13	6.6	25	13.1	198
Female	99	91.7	1	0.9	8	7.4	108
Total:	259	84.6	14	4.6	33	10.8	306
American Negroes:							
Male	30	33.3	18	20.0	42	46.7	90
Female	19	27.1	13	18.6	38	54.3	70
Total:	49	30.6	31	19.4	80	50.0	160

*These investigations were made possible through the cooperation of the authorities and staff of the public schools of San Francisco; the Albuquerque Indian School, New Mexico; and the Charity Hospital in New Orleans. I wish to especially thank Dr. Danforth and Dr. Ling of Stanford University, Palo Alto, California; Dr. Cummins of Tulane University, New Orleans, Louisiana; Dr. Bernard, director of the Charity Hospital, New Orleans, Louisiana; Mr. Hagberg, superintendent of the Indian Service, Albuquerque, New Mexico and Mr. Williams, principal of the Albuquerque Indian School.

grants from these localities.

Of the American Indians, with the exception of one Apache and six Pueblos, all were "full-blooded" Navajos, that is to say all their four grandparents were Navajo Indians. The Navajo tribe, which now have their "reservations" in the states of Arizona and New Mexico, had migrated to the South from the northern parts of Canada a few years before the Spanish conquest. There has been very little intermarriage with other tribes, and obviously none with the European settlers, though their former habit of raping women from the neighboring tribes, mostly Pueblos, may have transmitted a few genes of European (Spanish) origin.

The third group, the American Negro, surely is the most heterogeneous as to their racial heredity. They undoubtedly owe a considerable part of their genes to their European forefathers, but even their original African heredity must have been more or less influenced from non-Negroid admixture, especially from the Arabs.

Table I shows the frequencies of the different frontal hair directions within these different populations. In accordance with the previously published material it will be observed that the sex difference is of no significance regarding the various hair direction types. We will therefore confine our discussion to the three main population groups.

As Table I shows, the Chinese group has a strikingly low frequency of Type III, namely 5.2 percent, while on the other hand the recessive Type I is found in 90.2 percent, that is 9/10 of this population. The intermediate Type II was observed in 4.6 percent, and thus has but a slightly lower frequency than the strongest dominant Type III.

The Indian group does not differ much from the Chinese. Type II occurs exactly in the same percentage of 4.6. Type III shows a somewhat higher frequency, 10.8 percent, and Type I a correspondingly lower frequency of 84.6 percent. The Navajos, like the other American Indians certainly are a branch of the Asiatic (Mongoloid) race, and it is therefore not surprising that they also resemble each other in regard to the type of the hair slope. The higher frequency of Type III in the Indians may accordingly be ascribed to their supposed Spanish gene mixture.

However, the third group of the Table or the American Negro shows a remarkable contrast to the two Mongoloid groups. Among the American Negro, Type III occurs in 50 percent or exactly one-half of this population. Type I and Type II manifest themselves in 32.6 and 19.4 percent respectively. This is in good accordance with the distribution of the different types in the 42 mentally deficient American Negroes and "mulattos" previously examined[2]. They also showed the relatively high percentage of 46.5 of Type III, while the group, exclusive of the "mulattos", showed the even higher frequency of 58.0 percent of Type III.

As mentioned above the perviously published[1] data from family material analyses (pedigrees) are best explained by assuming that the genes producing the frontal hair direction types belong to a series of alleles. The gene responsible for Type III is designated D^{III} and the two others D^{II} and D^{I} respectively. As discussed earlier[1] there probably exists a high number of alleles within each of these major allele groups giving a nearly continuous variation of types of hair slope. In agreement with the theory of the skin growth centers[3], the skin growth from the nasal angles of the eyes competes with the growth directed from the crown.

In Table II the frequencies of these three major allele group are presented. There is a significant difference between the Negroid (African) race and the Mongoloid (Asiatic-American) regarding the distribution of these allele group frequencies. The data seem to justify the conclusion that the original Mongoloid man must have been homozygous for D^{I}, while the original Negroid may have had a high incidence of D^{III}.

The examined Northern Europeans[1] show intermediate values of allele frequencies with about 0.66, 0.19 and 0.15 for the alleles D^{I}, D^{II} and D^{III}. The other European groups hitherto examined are too small to permit reliable conclusions, but the data published[2] do not contradict our deductions as to the geographical centers of the different alleles: the Eastern Europeans seem nearer to the Asiatic, the Southern Europeans nearer to the African race in respect to the genes determining frontal hair direction.

It would seem that the geographic distribution of the different types of frontal hair slope in man affords a new means of tracing the migrations and intermixtures of the various racial groups.

TABLE II. Allele group frequencies.

Population	DI	DII	$DIII$
American Chinese	0.950	0.024	0.026
American Indians	0.920	0.025	0.055
American Negroes	0.55	0.15	0.29

Literature Cited

1. KIIL, VILHELM. *Jour. Hered.* 39: 206-16. 1948.
2. ———— *Jour Hered.* 39:281-85. 1948.
3. ———— *Jour. Exp. Zool.* 110:397-440. 1949.

VILHEIM KIIL
Oslo University, Norway

THE INHERITANCE OF GROWTH RATE AND EFFICIENCY OF GAIN

In Milking Shorthorn Steers

M. L. Kohli, A. C. Cook and W. M. Dawson*

ESTIMATES of the degree of inheritance of certain characters are considered to be helpful in adjusting the breeding program for livestock improvement, (Lush,[7] Wright,[13] Hazel[1] and Hazel and Terrill[2]). Relatively few studies of the degree of inheritance of various characters have been made for beef or dual-purpose cattle. Knapp and Nordskog,[4] in analyzing the records of 177 steers from 23 sires, estimated from paternal half-sib correlations the relative effect of heredity on final weight, daily gain, and efficiency of gain to be 81 99 and 75 percent, respectively. Heritability coefficients obtained from sire-progeny regression were 69 percent for final weight, 46 percent for daily gain, and 54 percent for efficiency of gain. After adjustments were made for the differences in feeding for the sires in each year, the heritability was found to be 94 percent for final weight, 97 percent for daily gain, and 48 percent for efficiency of gain. The authors mention that the causes of these high estimates were not known to them. Knapp and Clark,[5] in revising some of the estimates of heritability previously reported by them, state the new coefficients to be 86 percent for final feed lot weight (15 months) and 65 percent for gain in feed lot. They studied 110 sire groups in both cases. Patterson *et al.*,[9] using the records of 814 young bulls and 104 heifers, showed that the intra-year half-sib correlation of gain for bulls was +.260; for heifers, +.304. This high degree of relationship was considered by them to possibly be the result of favorable environmental conditions within sire groups, as compared with those between sire groups, although the small correlation between initial weight and gain (r =.063) seemed to show that it may not be too important.

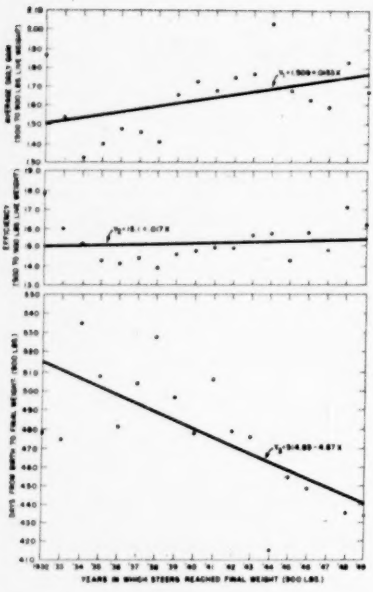
Estimates of heritability for average daily gain, days required to reach 900 pounds' live weight, and efficiency of gain for Milking Shorthorn steers are reported in this paper.

Material and Methods

The animals used in this experiment were 157 record-of-performance Milking Shorthorn steers bred and fattened at the Agricultural Research Center, Beltsville, Maryland, from 1932 to 1949, inclusive, as studied by Kohli *et al.*[6] They were the progenies of 125 dams and 29 sires. Most of the dams had only one steer offspring, 26 had two, and three had three. Of the 29 sires, six were purchased from different herds, while the other 23 were raised in the Beltsville herd. Each sire produced from one to 13 steers, 17 sires producing more than five.

During this period of 18 years, 91 of the first 94 steer calves were hand fed milk until they were weaned. The rest of the 157 steer calves were nursed from birth to weaning. Weaning was at 500 pounds' live weight except for the first 34 calves, which were weaned at 245 or 252 days of age. After weaning, the calves were full fed individually, in a record-of-performance test, from 500 to 900 pounds' live weight. The concentrates fed and the number of calves in each group were as follows: 35 calves received only No. 2 cracked yellow corn, with average total digestible nutrients (T.D.N.) of 80.6 percent; 26 calves received corn and varying proportions of a mixture of wheat, oats, linseed meal, bran, and corn; and 96 calves received a mixture of cracked yellow corn (5 parts), ground oats (3 parts), wheat bran (1 part), and linseed meal, 37 percent protein (1 part) by weight with T.D.N. of 75.5 percent and total digestible protein of 10.57 percent. Approximately one pound of No. 2 leavy alfalfa hay to two pounds of concentrate was given to all

*Guest research worker, Civil Veterinary Department, Punjab, India and Animal Husbandmen, Division of Animal Husbandry, U. S. Department of Agriculture, respectively. The authors wish to acknowledge the help of Dr. R. G. Schott, Bureau of Animal Industry, in reading and criticizing the manuscript.



AVERAGE GAIN

Figure 24

Average daily gain, efficiency of gain, and days required from birth to final weight of 157 Milking Shorthorn steers is shown above. The dots represent the mean values for the years in which steers reached final weight and were used in the calculation of the trend lines shown.

steers on the record-of-performance tests. None of the animals had access to pasture.

The steers were slaughtered at a live weight of as nearly 900 pounds as possible. The mean was 903 ± 0.82 pounds. The steer offspring of some sires were all slaughtered in one year, whereas those of other sires were slaughtered during several years, the maximum for any sire being five years. Usually the years in which a sire's offspring were slaughtered were consecutive but in some cases one or two years intervened. Efficiency was expressed as pounds of gains during the feeding period for each 100 pounds of total digestible nutrients consumed.

As a check on the results obtained with the 157 steers, which varied somewhat in the way they were handled, analyses were also made on 62 of these steers that varied the least in this respect. These 62 steers were all nursed from birth to weaning, were weaned at 500 pounds, and were fed the same mixed concentrate ration thereafter until they attained 900 pounds' live weight. The period in which they finished their tests (1943 to 1949) was shorter than the period for the 157 steers (1932 to 1949). Beginning in 1942, there was a definite attempt to obtain an equal number of steers (eight) from each sire and to make the cow herds to which the sires were mated more comparable than in the earlier part of the experiment. All the 62 steers were included in this part of the experiment.

In the absence of any records for the traits under study on the dams and sires used in the experiment, the estimates of heritability could be determined by only the paternal half-sib correlation method. Standard errors of the heritability coefficients were calculated as outlined by Hazel and Terrill.[2] Analysis of variance was used as described by Snedecor[10] and the theoretical components of variance determined according to the method of Winsor and Clarke[11] and of Hetzer *et al.*[3]

Results and Discussion

Sires used during the experiment varied from year to year. A few whose progeny performed well in the record-of-performance test were retained in the herd for several years and their sons were also used as sires. The effect of this selection, combined with the superiority of nursing to hand feeding of milk and the use of mixed concentrate instead of only corn in the latter period of the experiment, apparently caused a significant decrease in the average time required for a steer to reach a final weight of 900 pounds. On the other hand, the efficiency of gains during the test period and the average daily gain of the steers were increased slightly but not significantly, as shown by Kohli *et al*[6] and Figure 24. The regression line in the lower part of Figure 24 shows that in 18 years the decrease in time required to reach 900 pounds was 75 days. Effects of this decrease associated with years are shown in Tables I and II.* Although the steers were weaned and data on them obtained in different months of the year, analysis of variance showed the season to have little effect on any of these characters except on the time required to reach final weight, for which the effect of the month was significant at the five percent level. There was, however, some indication that this ap-

*The five Tables referred to herein are available as supplementary documents. To obtain this material order Document No. 3839 from the American Documentation Institute, 1719 N Street, N. W. Washington 5, D. C. Remit with order $1.00 for microfilm (images one inch high on standard 35 mm. motion picture film) or $1.00 for photocopies (6 × 8 inches) readable without optical aid.

parent seasonal effect was associated with differences in the sires. Thus the sires rather than the seasons may have affected the time required to reach final weight.

The results of analyses of variance between sire groups for 157 steers (Table I) showed that the mean squares for average daily gain, efficiency, and days to reach final weight were not significant when the variance for years and years and rations was removed. The mean squares are further analyzed into their theoretical components (Table II). S is the variance associated with differences between sires and E the remainder, including the environmental variance. The sum of E and S is the total or population variance when considered in an infinite population.

The parental half-sib correlation is the fraction $\frac{S}{E+S}$ (Table II). This value varies considerably depending on the analysis. Thus when it was calculated from the variance between sire progenies within rations and years it was .034 for average daily gain. When it was calculated from the variance between sire progenies without removing the variance for rations and years it was .333. The authors are inclined to believe that the best estimate of the paternal half-sib correlation furnished by these data is that determined from the intra-ration, intra-year variance between the sire progenies except where the variance between rations or years is not significant. This is the case with efficiency (third section of Table I) where the variance between rations was not significant when judged by the variance between years within rations. The best estimate for the paternal half-sib correlation for efficiency would then be the one calculated from the variance between sire progenies within years, or—.049 (Table II). Since it is theoretically impossible to have a negative value except by chance, it is assumed the correlation is actually zero.

Years, rations, method of handling prior to weaning, and sires were all confounded to a considerable extent in the data on the 157 steers. Therefore, results were obtained on 62 of the steers, which were handled in a more uniform manner, as already indicated. The results (Table III) show that there was not, on the average, a significant difference between sire progenies for any of the three characters studied, regardless of whether or not the variance between years is removed. Differences between years were significant for average daily gain but not for efficiency or days to final weight. Considerable difference was obtained between the paternal half-sib correlations for the 62 steers (Table IV) and those for the 157 steers. Following the same reasoning as before, the authors believe the variance between years should be removed for average daily gain but not for efficiency or days to final weight.

Since presumably with random mating the paternal half-sibs are on the average one-quarter less variable than the offspring of different sires—Wright,[12] Lush, [7, 8] and Hazel and Terrill[2]—it is possible to estimate the heritability of the characters due to the additive genetic variance by multiplying the half-sib correlations by four based on the techniques of the above-named investigators. This has been done for each of the characters for 157 steers and for the sub-sample of 62 steers using in each case the best estimate (author's judgement) of the paternal half-sib correlation available from the data (Table V). The heritability coefficients differ considerably for the 157 steers and for the 62 steers.

Such a result might be expected by chance with such large standard errors and so few steers per sire group (see values of S bottom of Tables I and III). Also it may be partially due to real differences in the samples. Any variance caused by differences in the dams mated to the different sires may have influenced the results. An attempt was made, particularly in the latter part of the experiment, to control this by mating each sire to a group of cows selected at random with age groups. An attempt was also made to have at least eight steers from each sire, and to prevent bias the first eight bull claves from a sire were castrated and put on test. Even with these precautions variations in the number of successful matings and in the sex ratio of calves from the better or poorer cows in the herd to which any bull was mated might be expected at times to vary enough with the number of matings usually made (20) to change the average of the steers from any one sire. Since all the 62 steers were from matings in which there were the above controls on the dams and since there was a greater control of the way in which the steers were handled, the authors believe the figures for the 62 steers are somewhat better than those for the 157 steers. However, it seems likely that the true values may lie between the two estimates obtained.

The heritability coefficient of 63.6 percent for average daily gain in the feed lot for the 62 steers agrees very closely with the revised figure of 65 percent obtained by Knapp and Clark.[5] On the other hand, the coefficient for efficiency, 25.6 percent, is only about half as large as the coefficient of 48 percent reported by Knapp and Nordskog.[4] Such comparisons are not strictly valid because of the differences in breeds, populations, and conditions but in planning breeding operations they may be of help in the absence of other data. Certainly if the lower values are closer to the true situation, it will be well to give more attention to progeny testing and family selection than if the higher values are more likely to be found.

Summary and Conclusions

A study was made of the inheritance of average daily gain, efficiency of gain in the feed lot, and days to reach 900 pounds' live weight of 157 purebred Milking Shorthorn steers, born and raised at the Agricultural Research Center, Beltsville, Maryland, 1932 to 1949, inclusive. In addition, results from a sample of 62 of these 157 steers which were more uniformly handled than the total sample were obtained. The degree of heritability of average daily gain, days to final weight, and efficiency of gain in the feed lot was estimated from the paternal half-sib correlations.

The best estimates, in the opinion of the authors, for the heritability in this herd of Milking shorthorns were:

Average daily gain in the feed lot, 500 to 900 pounds' live weight, 63.6 percent ± 62.0.

Efficiency of gain in the feed lot, 500 to 900 pounds' live weight, 25.6 percent ± 40.0.

Days required from birth to final weight (900 pounds' live weight) 3.6 percent ±33.2.

The above estimates are based on the sample of 62 steers.

In addition estimates are given based on the larger but more variable population of 157 steers.

Literature Cited

1. Hazel, L. N. *Genetics* 28: 476-490. 1943.
2. ———— and Clair E. Terrill. *Jour. An. Sci.* 4:347-358. 1945.
3. Hetzer, H. O., G. E. Dickerson and J. H. Zeller. *Jour. An. Sci.* 3:390-398. 1944.
4. Knapp, Bradford, Jr., and Arne W. Nordskog. *Jour. An. Sci.* 5:62-70. 1946.
5. ———— and R. T. Clark. *Jour. An. Sci.* 9:582-592. 1950.
6. Kohli, M. L., A. C. Cook and W. M. Dawson. *Jour. An. Sci.* 10:352-364. 1951.
7. Lush, J. L. *Empire Jour. Exp. Agric.* 3:25-30. 1935.
8. ————. *Amer. Soc. An. Prod. Proc.* pp. 293-301. 1940.
9. Patterson, B. E., J. H. Jones, J. J. Bayles and R. V. Turnbough. *Jour. An. Sci.* 8:808 (abstract). 1949.
10. Snedecor, George W. Statistical Methods. Iowa State Collage. Ames. 485 pp. 1948.
11. Winsor, C. P. and G. L. Clarke. *Jour. Marine Res.* 3:1-34. 1940.
12. Wright, Sewall. *Genetics* 6:111-178. 1921.
13. ————. *Amer. Soc. An. Prod. Proc.* pp. 18-26. 1939.

The Problem of The Pumpkin-Rind Watermelon

As far back as fifty years, or more, several strains of yellow-fleshed watermelons were common in certain parts of Oklahoma and Texas. They were usually outstanding for their delicious flavor and sweetness, as compared with most of the red-fleshed varieties. Toward the latter part of the 1951 watermelon season I accidentally stumbled across about a dozen vines of what might properly be called Pumpkin-Rind watermelons. These were growing in a field along the Red River valley within a hundred yards of the conventional red-fleshed varieties.

Enquiry of the owner as to their origin, elicited the statement that the seeds were sent him by "a woman in Texas." Further investigation disclosed that this woman was operating a seed house in "The Lone Star State" and that the seeds could be purchased in the regular way, under a certain trade-name.

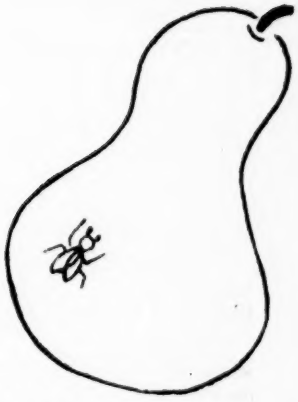
The owner and operator of this seed house reports that she does not know the origin of this very peculiar watermelon, but that it "has been around a good many years."

The outstanding character of The Pumpkin-Rind Watermelon, is its Golden Yellow color, when fully ripe. The young vines are not noticeably different in appearance from conventional varieties, but as they advance in growth and age, the vines and any younger watermelons slowly assume a yellow color. The vines gradually turn yellow from the crown outward and the young melons turn yellow as they ripen. The bright yellow color being a definite indication that the melons are fully ripe and the flesh, or meat is then of a deep-red color, comparing very favorably with that of other watermelons.

Just how such an unusual watermelon has been unnoticed by geneticists in high places, seems strange itself. Many theories have been advanced as to its origin; some suggest insect-crossing among red and yellow-fleshed varieties, others attribute it to mutation, or "sporting." Now, I am wondering if it was not a simple case of some "globe-trotter" bringing the seeds to Texas a few years ago, not realizing its potential importance.

Jay M. Gordon

Ervin Route,
Hugo, Oklahoma



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